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Activation of 2',5'-oligoadenylate synthetase and B-2 microglobulin in cancer patients treated with partially pure gamma interferon: Dependence of biological effect on administration route*

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Summary. Partially pure immune (gamma) interferon (IFN-gamma) was administered to patients intramuscularly (IM), by rapid IV bolus, and by 6-h continuous infusion as part of a phase I clinical trial. The activity of 2',5'-oligoadenylate synthetase (2,5 A) in peripheral blood cells and the concentration of beta-2 microglobulin (B-2M) in serum were monitored as indicators of interferon biological activity in vivo. Five patients received IFN-gamma by the IM route in doses ranging from 6.5×10^5 to 9.6×10^6 antiviral units daily. There was little induction either of serum B-2M or of 2,5A in peripheral blood cells. Eight patients received IFN-gamma by rapid (5 min) IV bolus infusion in doses ranging from 6.5×10^5 to 54×10^6 antiviral units daily. As with IM administration, there was little significant induction of 2,5A synthetase, but the concentration of B-2M was increased above pretherapy values in most patients. Eleven patients received IFN-gamma by 6-h infusion daily for 10 days at a dosage of 27×10^6 units/day. In contrast to IM and IV bolus administration, 6-h infusion of IFN-gamma resulted in significant induction of both B-2M serum concentration and of 2,5A activity in all patients. The induction of 2,5A was highest on days 2 and 4 of therapy and decreased to pretherapy values by day 7. During the second 10-day course of the infusion study 2,5A activity was not induced until day 7 of therapy, and it decreased rapidly thereafter. These studies show clearly that consistent biological activity such as B-2M activation and specific intracellular biochemical events such as 2,5A induction may be optimally obtained by the administration of IFN-gamma by continuous IV infusion.

Introduction

The interferons are a class of peptide hormones with antiproliferative and immunoregulatory properties [1]. The family of interferons has been divided into distinct categories based on studies of their physiochemical properties and their biological activites. Leukocyte interferon (IFN-alpha) and fibroblast interferon (IFN-beta) are produced by cells after exposure to viruses or double-stranded RNA and have been shown to share a common surface receptor. Clinical studies have shown that IFN-alpha and IFN-beta have induced tumor regression in a variety of malignancies [5, 6, 9, 11]. Immune interferon (IFN-gamma) is produced by T lymphocytes in response to antigen or mitogenic stimulation [18]. Recent research has shown that both partially purified and pure IFN-gamma have direct growth-inhibitory activity in vitro [15, 22]. IFN-gamma has been shown also to bind to a cell surface receptor distinct from that of IFN-alpha or IFN-beta. In addition, the spectrum of biological action and intracellular mechanism of action for IFN-gamma may differ from that of the other interferons [14, 23]. Studies have shown that, like IFN-alpha and IFN-beta, IFN-gamma can directly induce the intracellular activity of the enzyme 2',5'-oligoadenylate synthetase (2,5A) [4, 8]. Schattner et al. [16] have suggested that 2,5A activity may reflect the biological activity of interferon in vivo. In addition, the serum level of B-2 microglobulin (B-2M) has been found to be increased in patients undergoing interferon therapy [22]. Accordingly, we monitored B-2M serum concentrations and 2,5A activity in peripheral blood cells isolated from patients treated with partially pure IFN-gamma to determine the best route of administration and the dosage required to induce these biological activities in vivo.

Materials and methods

Materials

Partially purified IFN-gamma was obtained from Meloy Laboratories (Springfield, Va). Detailed methods of production and purification are reported elsewhere [2, 3]. The interferon for clinical use was provided in vials containing 6×10^5 to 4×10^6 units and stored as a liquid at 4 °C.

Patient selection and experimental design. Twenty-four patients with advanced metastatic cancer were studied. The patients were ambulatory and had not received therapy for at least 4 weeks before the study began. All patients signed informed consent forms according to institutional guidelines. Clinical investigation studies were approved by the Bureau of Biologics of the Food and Drug Administration.

The first five patients were given IFN-gamma IM into the deltoid muscle. Individual doses of 1.2×10^5, 2.4×10^6, 4.8×10^6, and 9.6×10^6 units were administered to each patient on a daily schedule.

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The next eight patients were entered in the IV phase of the study. Doses ranged from 1.5×10^5 to 54×10^6 units administered in 100% increments and were infused over a 5-min period directly into the antecubital vein. Intervals of 72 h to 96 h elapsed between doses.

In the continuous infusion phase of the study, IFN-gamma was administered to 11 patients as follows: a loading dose of 3×10^6 units was given IV bolus injection over 5 min, followed by an infusion of IFN-gamma at a rate of 6×10^6 units hourly for 6 h. The first 2 patients entered in the study received 6-h infusions for 5 consecutive days. The next 9 patients received 6-h infusions for 10 consecutive days. The results of this clinical trial have been reported elsewhere [7].

Cultured cells. Daudi cells, a human lymphoblastoid cell line, was used as a 2,5A synthetase source for enzyme standard in all experiments. The cells were maintained in RPMI medium supplemented with 10% heat-inactivated fetal calf serum (KC Biological, Lexena, Kansas), gentamicin, and L-glutamine. Daudi cells were subcultured three times weekly to maintain a density of between 0.2 and 1.0×10^6 cells/ml.

Peripheral blood cells (PBCs). Patient PBCs were separated and collected from a standard continuous Ficoll-hypaque gradient. The cells were washed and diluted to the desired density with Dulbecco's phosphate-buffered saline (PBS) (GIBCO, Grand Island, NY) containing 1 mg/ml fraction V bovine serum albumin (Sigma, St. Louis, Mo). For analysis of 2,5A activity, PBCs were washed with PBS, transferred to plastic 400-ml microcentrifuge tubes, and stored as cell pellets at $-80\,^{\circ}C$ until analyzed.

2,5A Assay. The 2,5A assay is a modification of the procedure described by Minks et al. [12]. Frozen PBC pellets obtained from chronic myelogenous leukemia patients were suspended in 80 µl hypotonic buffer containing 10 m*M* KCl, 1,5 m*M* Mg $(OAc)_2$, 20 m*M* Hepes buffer (pH 7.4), and 0.5% (v/v) Triton X-100. The cell suspensions were frozen and thawed three times in liquid N_2 to insure cellular disruption. A 40 µl aliquot of hypotonic buffer containing 50% glycerol was added, and the cell lysates were centrifuged briefly (Fisher centrifuge model 235A) at 13,000 *g*.

The 2,5A assay incubation mixture contained 120 m*M* KOAc, 25 m*M* fructose-1,6-diphosphate, 1 m*M* dithiothreitol, 0.2 mg/ml Poly (I-C), and a 200,000-cpm sample of [2,8^{3}H]-ATP (29 Ci/mol) in a total volume of 50 µl.

For the assay, 20 µl cell lysate and 30 µl of this incubation mixture were combined in 1.5-ml microcentrifuge tubes, and the samples were incubated at 30 °C for 20 h. The reaction was stopped by heating the samples to 95 °C for 3 min.

To analyze the reaction products, the samples were diluted to 1 ml with a buffer (buffer G) containing 90 m*M* KCl and 20 m*M* Hepes buffer, pH 7.4, and applied to minicolumns of DE-52 that had previously been equilibrated with buffer G (bed size 1×1.2 cm). The columns were washed with 24 ml buffer G to remove unreacted [^{3}H]ATP, and the tritiated oligomers were eluted directly into scintillation vials with 2 ml of buffer containing 0.35 *M* KCl and 20 m*M* Hepes (pH 7.4). Liquiscint scintillation fluid (National Diagnostics, Somerville, NJ) 15 ml was added to each vial, and the dpm counted in a Packard 3385 Tricarb Scintillation Counter. All samples were assayed in triplicate. Protein concentration in the cell lysate was measured in triplicate using the Bio-Rad Protein Assay Kit. Values for enzyme activity were expressed as nanomoles of ATP incorporated into product per milligram of protein (from means of triplicates). In this assay, the intra-assay and the interassay variability of results were 2.95% and 3.71% respectively.

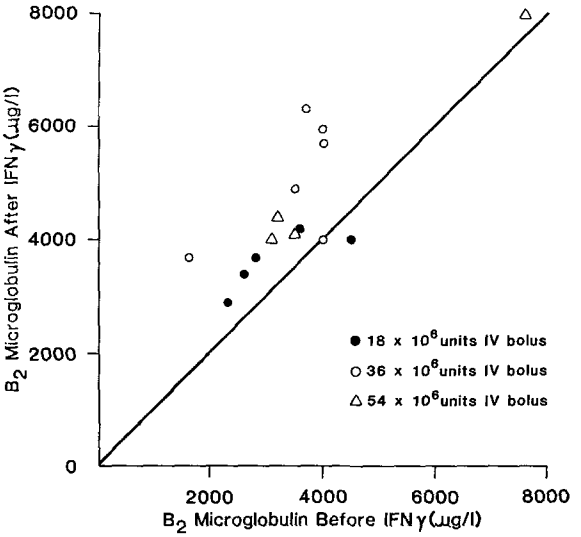

Fig. 1. Serum concentration of B-2M before and after daily administration of various doses of IFN-gamma by rapid IV bolus

Results

B-2M Serum concentration

The concentration of B-2M in serum of patients after administration of IFN-gamma by IV bolus, IM, or by 6-h continuous infusion are shown in Figs. 1, 2, and 3, respectively. Intramuscular administration caused little induction of B-2M serum levels over pretherapy values. In most patients who received IV bolus doses of IFN-gamma, there

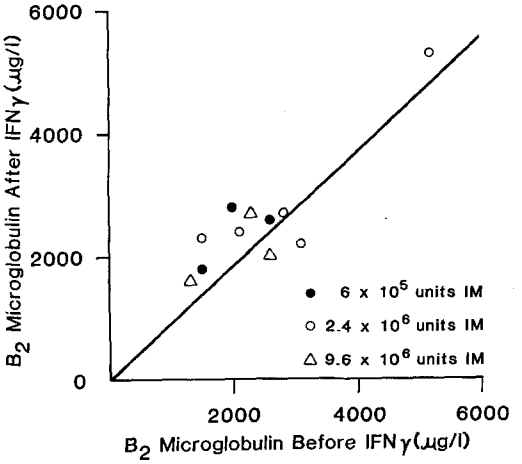

Fig. 2. Serum concentration of B-2M before and after daily administration of various doses of IFN-gamma by the IM route

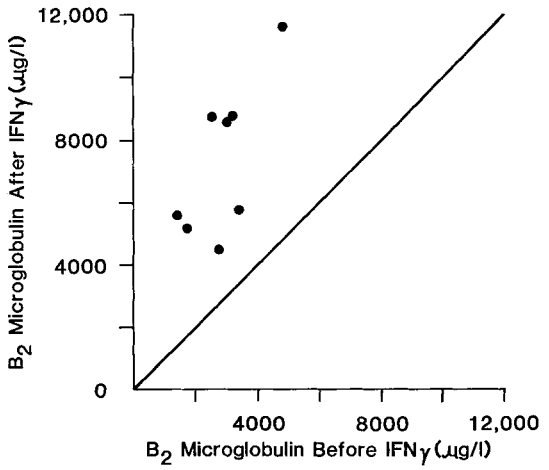

Fig. 3. Serum concentration of B-2M before and after daily administration of IFN-gamma by 6-h continuous infusion

was some induction of activity compared to pretherapy values. After continuous infusion of IFN-gamma, however, the serum B-2M increased significantly in all patients studied.

2,5A Activity

As Fig. 4 shows, the activity of 2,5A in the patients' PBCs after IV Bolus administration was slightly elevated above pretherapy values on day 7 of therapy, but the increase

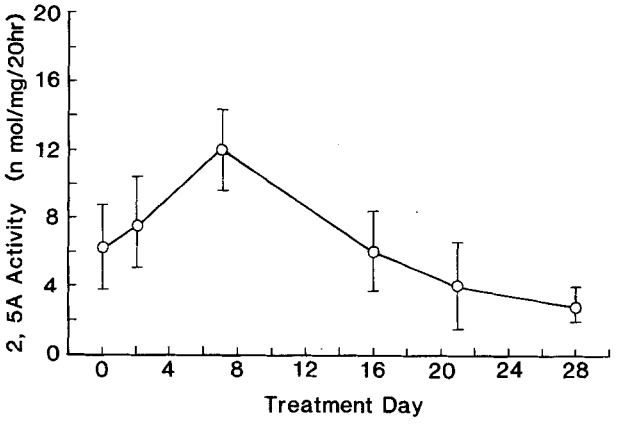

Fig. 4. Activity of 2,5A in peripheral blood mononuclear cells during daily IV administration of IFN-gamma

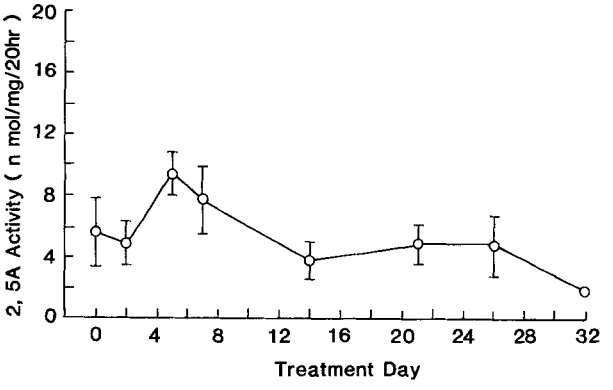

Fig. 5. Activity of 2,5A in peripheral blood mononuclear cells during daily IM administration of IFN-gamma

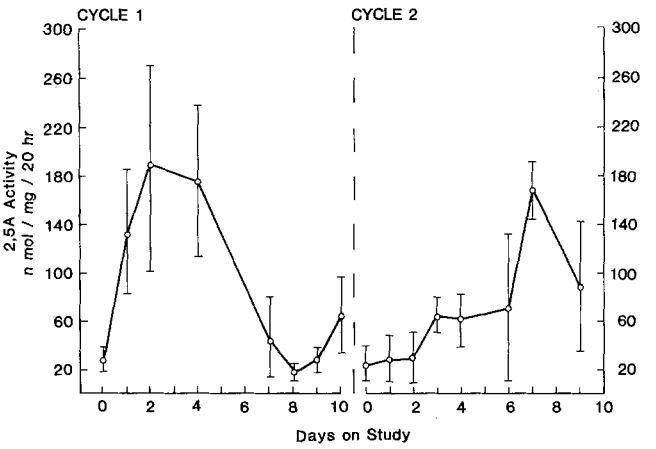

Fig. 6. Activity of 2,5A in peripheral blood mononuclear cells during treatment with two 10-day courses of daily administration of IFN-gamma by 6-h continuous infusion

was not statistically significant. Similarly, after IM administration, 2,5A activity was elevated slightly on days 5 and 7 of therapy (Fig. 5), but this increase was also not statistically significant. In contrast, the mean 2,5A activity in PBCs obtained from patients after continuous IV infusion (Fig. 6) increased from a pretherapy values of 30 nmol/mg to approximately 190 nmol/mg (6-fold increase) by 48 h after initiation of therapy. Despite continued therapy, 2,5A activity fell to approximately pretherapy values by day 7 of therapy. Cycle 2 of therapy began after a 2- to 3-day rest period from the previous cycle. The activity of 2,5A remained low in these patients early in the cycle until about the 6th or 7th day of therapy, when 2,5A activity increased temporarily.

Discussion

The biochemistry of interferon action has been the subject of many studies [10, 17, 19]. The intracellular antiproliferative and antiviral actions of the interferons appear to be mediated, at least in part, by the induction of 2,5A, resulting in activation of latent endonuclease activity and a protein kinase (PK) responsible for the phosphorylation and inactivation of peptide chain initiation factor (EIF-2). The induction of both the 2,5A and PK activities has been found to require both de novo protein synthesis and the presence of double-stranded RNA [21]. The kinetics, specificity, and dose-dependence of 2,5A induction by IFN in both human and animal cells in culture have been well-established [10, 21]. Schattner et al. [16] have shown that peripheral mononuclear cells obtained both from normal volunteers and from cancer patients demonstrate induction of 2,5A activity after in vitro exposure to IFN. These studies have shown also that 2,5A activity in PBCs may be a useful marker of in vivo IFN activity. In a previous study [13], we also showed that in patients with CML treated with IFN-alpha, the induction of 2,5A activity in peripheral blood cells was correlated with in vivo response to therapy.

In the current study, we demonstrate that there is little induction of 2,5A synthetase or B-2 microglobulin with IM or with bolus IV administration of IFN-gamma. We have previously shown (24) that partially pure IFN-gamma is poorly absorbed into the vasculature after IM administra-

tion. Therefore, the poor absorption of IFN-gamma after IM administration may account for the failure to induce either 2,5A activity or serum B-2M. In addition, after bolus administration, IFN-gamma antiviral activity was found to be cleared rapidly (serum $T_{1/2}$ between 3 and 34 min) from the circulation and may therefore be unavailable for the requisite prolonged contact time for activation of target cells by IFN-gamma (25) also precluding activation of B-2M or 2,5A activity. Continuous 6-h infusion of IFN-gamma resulted in significant induction of 2,5A activity and B-2M after the start of the 10-day therapy course. This suggests that the pharmacological difficulties posed by the poor absorption of the IM route, and the rapid clearance after IV bolus administration may be circumvented by continuous IV venous infusion, thus permitting greater expression of in vivo biological activity of IFN-gamma. However, the induction of 2,5A activity appeared to be transient despite continued administration of IFN-gamma. In the second course of therapy, induction of 2,5A activity did not occur until late in the therapeutic course. This suggests that a refractory period may occur in PBCs in vivo after treatment with IFN-gamma. A recovery period of approximately 10 days may therefore be required for recovery of the biological response potential. A variety of reasons might account for this observed refractory period. One possibility is that binding of IFN-gamma to cells may cause down-regulation or loss of receptors on target cells similar to that found for IFN-alpha [13] and that the observed refractory period may be required for receptor synthesis and re-expression.

In summary, this study shows clearly that IM and bolus IV administration do not result in in vivo activation of 2,5A activity in peripheral blood cells or B-2 microglobulin in serum. A 6-h continuous infusion was found to produce a significant induction in both 2,5A activity and B-2M serum concentration. However, the 2,5A response appeared to be transient despite daily administration of the agent by continuous infusion, which suggests that a refractory period follows the 2,5A response. This suggests that IFN-gamma should be given by IV continuous infusion for periods of only 5–6 days, followed by a rest period to allow recovery of biological response potential.

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