Radiol Oncol 1993; 27: 286-92.

Antitumor effect of interferon-α administered by different routes of treatment

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Besides the fact that interferons were identified as factors capable of inhibiting viral infections, they have proved to be antiproliferative, immunomodulatory and differentiation-inducing factors. On the basis of these activities, they have been employed clinically for treatment of various tumors. The study was performed to determine whether there was different antitumor effect of recombinant human interferon-α A/D (rHuIFN-α A/D) when it was given as a local or systemic therapeutical agent. Two different tumor models, i.e. subcutaneous (s.c.) and intraperitoneal (i.p.) B-16 melanoma on C57Bl/6 mice, were employed in these experiments. Experimental mice were treated locally or systemically with different doses of rHuIFN-α A/D; the treatment was begun 24 hours after tumor cell inoculation and continued through five consecutive days. Intraperitoneal treatment of animals with i.p. tumors resulted in significantly longer survival time in comparison with control group or with subcutaneously treated animals ($p<0.001$). Similarly, the delay of tumor detection and tumor growth in mice with s.c. tumors treated subcutaneously with rHuIFN-α A/D was significantly greater than in intraperitoneally treated animals ($p<0.01$). According to these results we can conclude that rHuIFN-α A/D is much more potent antitumor agent when it is used locally. However, systemic treatment with higher doses was effective in both tumor models and it is still more convenient for treatment of some tumor lesions which are not accessible for local treatment.

Key words: melanoma, experimental-drug therapy; interferon-alpha; drug administration routes

Introduction

Interferons are glycoproteins which were identified as factors capable of inhibiting viral infections.[1, 2] Besides, interferons have proved to be antiproliferative, immunomodulatory and differentiation inducing factors.[3, 4, 5] Other putative functions include antioncogene activity and mobilisation of energy stores during sickness.[6,7]

Three subtypes of interferons (IFN α, β and γ) have been identified, differing in terms of their cell surface receptors, their acid stability, their primary sequence and their chromosomal location and organisation.[3, 8] Interferon-α and interferon-β produced by leukocytes and fibroblasts, respectively, are acid stable and share the same receptor, while interferon-γ is produ-

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UDC: 616-006.81-085

ced by T lymphocytes, is acid labile and has a different receptor.[4, 8]

The precise mechanisms of action for the antitumor effects of interferons are not fully explained. They involve both direct (antiproliferative effects, cytotoxic effects and enhancement of cell surface antigen expression on tumor cells) and indirect antitumor action (activation of macrophages/monocytes, activation of T cells, activation of NK cells and modulation of antibody production).[9, 10]

More then 20 subtypes of interferon-α are known, but only few of them are used systemically or locally in the treatment of neoplasms as hairy cell leukemia, AIDS - related Kaposi sarcoma, Hodgkin's disease, non - Hodgkin's lymphomas, oral cancer, malignant melanoma, renal cell carcinoma and bladder cancer.[11-16]

In our experiments we investigated the relative capability of local versus systemic treatment with rHuIFN-α A/D as an antitumor agent against B-16 melanoma. To address this question we used two different tumor models: i.p. and s.c. B-16 melanoma tumors.

Materials and methods

Animals

Six to eight weeks old female C57Bl/6 mice were used in the experiments. Mice were purchased from Jackson Laboratories (Bar Harbor, USA) and held in a pathogen free animal colony. The adaptation period before use was two to three weeks. At least nine healthy animals with normal body weight were included in each experimental group.

Tumor models

Subcutaneous (s.c.) and intraperitoneal (i.p.) tumors were employed as tumor models. Subcutaneous tumors were induced subcutaneously in the left lower abdomen with 10^6 B-16 melanoma cells in 0.1 ml EMEM (Eagle's minimal essential medium) supplemented with 2% fetal calf serum (FCS), while mice for i.p. tumors were inoculated with the same number of viable cells intraperitoneally. In the experiments with s.c. tumors the day of tumor detection was monitored and tumor growth was followed by measuring two tumor diameters with a vernier caliper. The tumor burden was calculated by the standard formula for a prolate sphere $V = \pi/6 \times d_1 \times d_2^2$ ($d_2 < d_1$). Mice with i.p. tumors were monitored for the day of death and the increased life span (ILS) was calculated. Also, mice with s.c. tumors were monitored for the day of tumor development and the increased tumor detection span (ITDS) was determined as shown below.

$$\text{ILS} = \frac{\text{av. day of death for IFN treated mice - av. day of death for control}}{\text{average day of death for control}} \times 100$$

$$\text{ITDS} = \frac{\text{av. day of tu. det. for IFN treated mice - av. day of tu. det. for control}}{\text{average day of tumor detection for control}} \times 100$$

Tumor cells

Murine B-16 melanoma cells (clone F1)[17] were grown in EMEM supplemented with 10% FCS, penicillin (100 units/ml), streptomycin (100 μg/ml) and gentamycin (11 μg/ml). The final cell suspension for inoculation (10^6 viable tumor cells per 0.1 ml) was prepared with EMEM supplemented with 2% FCS and antibiotics as indicated above.

Interferon

Recombinant human interferon-α A/D (rHuIFN-α A/D) used in this study was generously provided by Dr. Michael Brunda of Hoffman-La Roche (Nutley, New Jersey) and had a specific activity of 6.4×10^7 U/mg of protein. Recombinant human IFN-α A/D is a recombinant molecular hybrid of two subtypes of HuIFN-α which exerts antiviral, antitumor and myelotoxic activities in mice.[18,19] The interferon was diluted in phosphate buffered saline (PBS) supplemented with 0.3% bovine serum albumin (BSA, Sigma Chemical Company) and frozen at -70°C until used for treatment.

Treatment

Interferon treatment was started 24 hours after tumor cell inoculation and continued daily through five consecutive days. Animals with i.p. tumors were treated with different IFN doses (3000 U/0.2ml, 10000 U/0.2ml or 30000 U/0.2ml per animal per day) intraperitoneally (locally) or subcutaneously (systemically). Subcutaneous tumor bearing animals were treated with the same doses as mentioned above, but in this case subcutaneous treatment was performed as local and intraperitoneal as systemic treatment. The animals in the control group were injected subcutaneously or intraperitoneally with 0.2 ml of PBS supplemented with 0.3 % BSA.

Statistical analysis

The data were evaluated for significance using Student's T-test.

Results

Experiments were performed to determine whether the antitumor effect of rHuIFN-α A/D was different when the agent was administered locally or systemically. Two different tumor models were employed: s.c. B-16 melanoma and i.p. B-16 melanoma.

Antitumor effect on s.c. tumors

Mice were inoculated s.c. with B-16 melanoma tumor cells and randomly divided in eight groups:

– control group treated subcutaneously with PBS/BSA;

– control group treated intraperitoneally with PBS/BSA;

– group treated subcutaneously with 3000 U (3 KU) of rHuIFN-α A/D;

– group treated intraperitoneally with 3000 U (3 KU) of rHuIFN-α A/D;

– group treated subcutaneously with 10000 U (10 KU) of rHuIFN-α A/D;

– group treated intraperitoneally with 10000 U (10 KU) of rHuIFN-α A/D;

– group treated subcutaneously with 30000 U (30 KU) of rHuIFN-α A/D and

– group treated intraperitoneally with 30000 U (30 KU) of rHuIFN-α A/D.

Table 1. Average day of tumor detection for s.c. B-16 melanoma bearing mice treated subcutaneously or intraperitonealy with rHuIFN-α A/D.

	Average day of tumor detection	SD*	p-value (comparing to the control)	p-value (comparing the same doses)
i.p. control	9.9	2.1	-----	-----
i.p. 3 KU	12.7	2.9	0.0039	-----
i.p. 10 KU	12.2	2.5	0.0087	-----
i.p. 30 KU	14.7	4.8	0.001	-----
s.c. control	10.4	2.2	-----	-----
s.c. 3 KU	16.9	5.2	0.0001	0.005
s.c. 10 KU	19.9	3.9	0.0001	0.0001
s.c. 30 KU	20.2	3.8	0.0001	0.001

*SD - Standard deviation

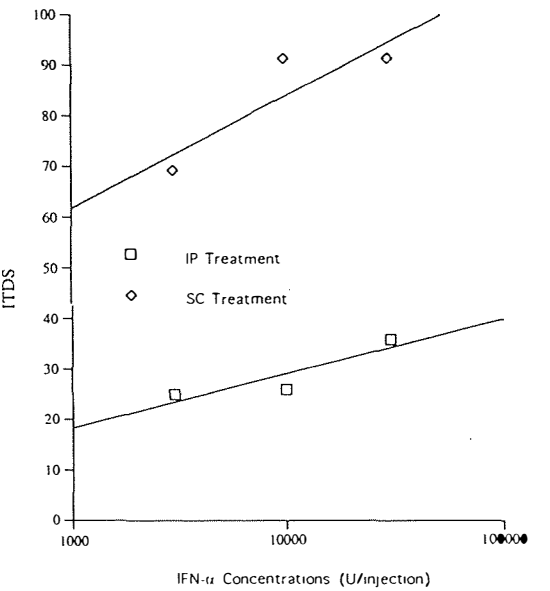

Figure 1. Increase in tumor detection span (ITDS) for subcutaneous B-16 melanoma tumors. Tumors were implanted using 10^6 viable tumor cells, and 24 hours later treated subcutaneously or intraperitoneally with different concentrations of rHuIFN-α A/D during five consecutive days.

Starting 24 hours after tumor cell inoculation and continuing for five days, mice were injected s.c. or i.p. with either rHuIFN-α A/D (different concentrations) or PBS/BSA.

The day of tumor detection and tumor growth were monitored. Table 1 presents the average results of two identical experiments that gave similar results. Both subcutaneous (local) and intraperitoneal (systemic) treatments caused a significant antitumor effect at all interferon concentrations employed. However, it can be seen that local treatment was more effective than systemic treatment.

Locally treated mice with 3 KU of rHuIFN-α A/D developed tumors in 16.9 days on average, with 10 KU in 19.9 days, and with 30 KU in 20.2 days; these periods being 62.5%, 91.3% and 94.2% longer than those in the control group (Table 1, Figure 1).

Systemically treated mice with 3 KU developed tumors in 12.7 days in average, with 10 KU in 12.2 days, and with 30 KU in 14.7 days; those periods being 28.3%, 23.2% and 48.5% longer than those in the control group (Table 1, Figure 1). Tumor growth kinetics was the same as in the control group, while local treatment slowed down the tumor growth in all treated groups (Figure 2).

Statistically significant differences in the day of tumor detection were observed between the two routes of interferon administration for all treatment doses ($p = 0.005$ with 3 KU, $p = 0.0001$ for 10 KU, and $p = 0.001$ for 30 KU of rHuIFN-α A/D).

Antitumor effects on i.p. tumors

It was important to consider previous data from s.c. tumor model in order to asses whether the differential responsiveness of the tumors would be observed on i.p. tumor model after different routes of treatment with rHuIFN-α A/D. To address this point, mice were inoculated i.p. with B-16 melanoma tumor cells and randomly distributed (as mice with s.c. tumors), into eight groups. Mice were also treated locally (intraperitoneally) and systemically (subcutaneously) with different doses of rHuIFN-α A/D or PBS/BSA. Treatment schedule was the same as the one described above for s.c. tumors. Mice were monitored for the day of death.

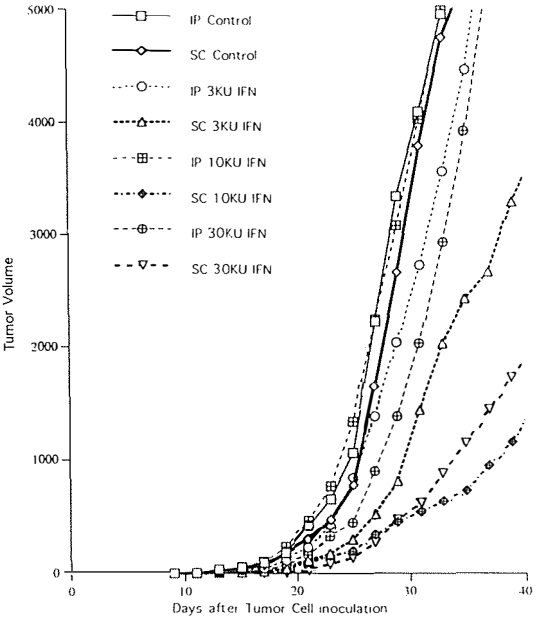

Figure 2. Growth kinetics of subcutaneous B-16 melanoma implanted in the left lower abdomen using 10^6 viable tumor cells, and treated subcutaneously or intraperitoneally with rHuIFN-α A/D.

Table 2 presents the average results of two identical experiments which gave similar results. It can be seen that also in i.p. tumors local treatment was more effective than systemic

Table 2. Average day of death for i.p. B-16 melanoma bearing mice treated subcutaneously or intraperitonealy with rHuIFN-α A/D.

	Average day of death	SD*	p-value (comparing to the control)	p-value (comparing the same doses)
s.c. control	19.9	1.8	-----	-----
s.c. 3 KU	19.6	1.7	0.5688	-----
s.c. 10 KU	20.9	1.7	0.0714	-----
s.c. 30 KU	21.5	1.8	0.0098	-----
i.p. control	19.3	1.3	-----	-----
i.p. 3 KU	22.6	2.5	0.0001	0.0001
i.p. 10 KU	23.3	1.9	0.0001	0.0004
i.p. 30 KU	24.8	3.1	0.0001	0.0002

*SD - Standard deviation

treatment (Table 2, Figure 3). Percent of increase in life span (%ILS±SE) for locally treated mice with rHuIFN-α A/D was 17.3±2.99 (3 KU), 20.5±2.27 (10 KU) and 28.7±3.68 (30 KU); for systemically treated mice the %ILS was -1.7±1.96 (3 KU), 5.2±1.98 (10 KU) and 7.9±2.08 (30 KU) (Figure 4). Owing to a higher agressivness of i.p. tumors, systemic (subcutaneous) treatment did not statistically significantly affect the average day of death in comparison with control mice, except when the mice were treated with the highest dose (30 KU) (Table 2, Figure 3).

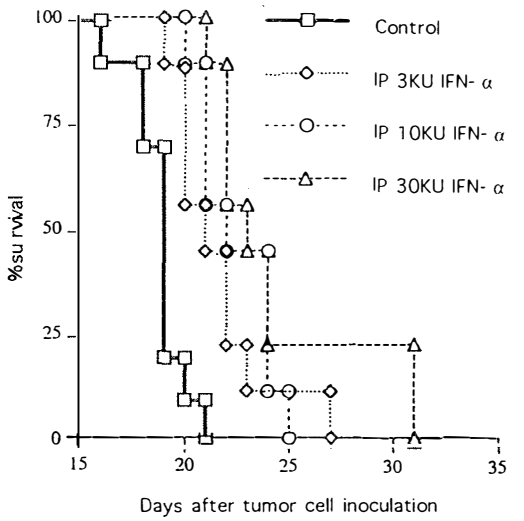

Figure 3. Survival curves for intraperitoneal B-16 melanoma bearing mice (C57Bl/6); tumors were induced intraperitoneally with 10^6 viable tumor cells and 24 hours later treated intraperitoneally (upper figure) or subcutaneously (lower figure) with different concentrations of rHuIFN-α A/D during five consecutive days.

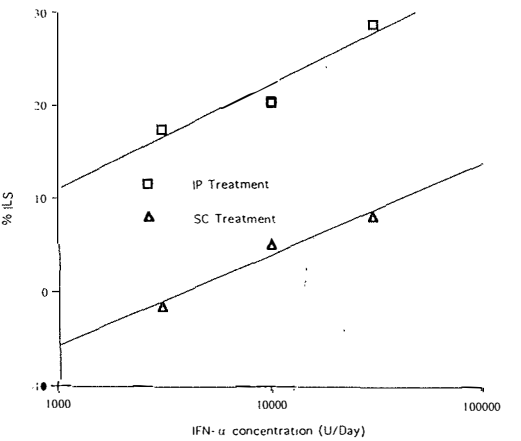

Figure 4. Increase in life span (ILS) for intraperitoneal B-16 melanoma bearing mice treated systemically or locally with rHuIFN-α A/D.

However, statistically significant increase in life span was observed when we compared locally (intraperitoneally) treated animals to the ones treated systemically (subcutaneously) with the same dose of rHuIFN-α A/D; the p-value for mice treated with 3 KU was 0.0001, for mice treated with 10 KU 0.0004 and for mice treated with 30 KU 0.0002. Moreover, the animals that received a threefold lower dose (10 KU) of rHuIFN-α A/D locally (intraperitoneally) survived significantly longer than those treated systemically (subcutaneously) with 30 KU ($p = 0.005$).

Based on the results obtained in both tumor models, it is clear that maximal antitumor activity occurred when rHuIFN-α A/D was given locally. Systemic treatment was moderately effective: more effective in s.c. tumors when rHuIFN-α A/D was administered intraperitoneally than on i.p. tumors when it was administered subcutaneously.

Discussion

Previous experimental findings demonstrated that IFN-α has reproducible antiproliferative effects in vitro[20-22] and in *vivo*.[23] On the basis of these findings, IFN-α has been employed clinically for treatment of various tumors.

Today, IFN-α is approved as an antiproliferative agent for the treatment of hairy cell leukemia and AIDS related Kaposi sarcoma.[5,10] Nevertheles, IFN-α as a single agent has been reported to induce clinical remission in many hematological malignancies and solid tumors.[16,24,25] Moreover, IFN-α has reproducible activity against malignant melanoma, a tumor for which conventional chemotherapy has poor efficacy.[10,25]

The present study was undertaken to assess which route of administration is more suitable for IFN-α treatment. Therefore, we chose s.c. and i.p. B-16 melanoma tumor models. Mice were treated locally and systemically for five consecutive days with different doses of rHuIFN-α A/D. Systemic treatment of s.c. tumors was performed in the form of intraperitoneal injection, while locally treated animals were injected subcutaneously. In contrast, in the i.p. tumor model intraperitoneal administration was performed as a local and subcutaneous as a systemic treatment. In both cases local treatment proved to be significantly superior to systemic. An interesting observation was that systemic (intraperitoneal) treatment of s.c. tumors resulted in a statistically significant delay in tumor detection at all interferon concentrations examined, while systemic treatment (subcutaneous) of i.p. tumors did not significantly increase the life span of treated animals (except 30 KU). The fact that developed tumors in systemically treated animals continued growing at the same rate as tumors in control mice, suggests that systemically administered rHuIFN-α A/D exerts antitumor effect only on a very small tumor burden.

In accordance with our observations, rHuIFN-α A/D is more effective when given as a local therapeutical agent. Nevertheless, when we have to use rHuIFN-α A/D systemically, it is much more advisable to administer it intraperitoneally than subcutaneously. This is also in agreement with previous pharmacokinetic findings that intraperitoneal administration of IFN-α has good bioavailability (30 times higher) compared to the intravenous route.[26]

The role of IFN-α in the treatment of malignancies has not yet been fully established. Many questions remain to be answered concerning the optimal strategy for incorporating IFN-α into anticancer therapy, and one of them is the optimal route of its administration. However, the future of IFN-α usage in oncology seems to be in its local (and also systemical) use as adjuvant therapy after the tumor burden has been reduced by other therapeutic modalities.

Acknowledgement

This work was supported by U.S. Public Health Service Grant (National Cancer Institute).

References

1. Isaacs A, Lindenmann J. Virus interference. I. The interferon. *Proc R Soc Lond Ser B* 1957; **147:** 258-67.
2. Lindenmann J. Induction of chick interferon: procedures of the original experiments. *Methods Enzymol* 1981; **78:** 181-8.
3. Friedman MR, Vogel NS. Interferons with special emphasis on immune system. *Adv Immunol* 1983; **34:** 97-140.
4. Baron S *et al.* The Interferons. Mechanisms of action and clinical applications. *JAMA* 1991; **266:** 1375-83.
5. Elsdssr-Beile U, von Kleist S. Cytokines as therapeutic and diagnostic agents. *Tumor Biol* 1993; **14:** 69-94.
6. Kurzrock R, Talpaz M, Gutterman JU. Interferons α, β, γ: basic principles and preclinical studies. In: DeVita VT Jr., Hellman S, Rosenberg A eds. *Biological therapy of cancer.* New York: J.B. Lippincott Co. 1991: 247-75.
7. Patton JS *et al.* Interferons and tumor necrosis factors have similar catabolic effects on 3T3L1 cells. *Proc Natl Acad Sci USA* 1986; **83:** 8313-8.
8. Itri ML. The interferons. *Cancer* 1992; **70** (Suppl 4): 940-5.

9. Fleischmann WR Jr., Fleischmann CM. Mechanisms of interferons antitumor action. In: Baron S *et al* eds. Interferon: Principles and medical applications. Galveston: UTMB 1992: 299-309.

10. Dorr RT. Interferon-α in malignant and viral diseases. *Drugs* 1993; **45(2):** 177-211.

11. Rao SV, Wadler S. Curent use of interferons. *Contemp Oncol* 1992; **3:** 44-9.

12. Kuo JY *et al.* Impaired interferon-α production in whole blood cultures from bladder cancer patients. *Urol Res* 1991; **19:** 51-6.

13. Rassiga-Pidot AL, McIntyre OR. In vitro leucocyte interferon production in patients with Hodgkin's disease. *Cancer Res* 1974; **34:** 2995-3002.

14. Ho AD, Moritz T, Rensch K, Hunstein W, Kirchner H. Deficiency in interferon production of peripheral blood leucocytes from patients with non-Hodgkin lymphoma. *J Interferon Res* 1988; **8:** 405-13.

15. Jamkar AV, Banerjae AC, Gore MM, Sathe PS, Ghosh SN. Interferon producing capacity of peripheral mononuclear cells in oral cancer patients. *Indian J Cancer* 1989; **26:** 76-84.

16. Wadler S. The role of interferons in the treatment of solid tumors. *Cancer* 1992; **70** (Suppl 4): 949-58.

17. Fidler IJ. Selection of successive tumor lines for metastasis. *Nature New Biol* 1973; **242:** 148-9.

18. Kramer MJ *et al.* Cell and virus sensitivity studies with recombinant human alpha interferons. *J Interferon Res* 1983; **3:** 425-35.

19. Rosenthal GJ *et al.* Organ-specific hematopoetic changes induced by a recombinant human interferon alpha in mice. *Fundam Appl Toxicol* 1990; **14:** 666-75.

20. Paucker K, Cantell K, Henle W. Quantitative studies of viral interference in suspended L cells, III. Effect of interfering viruses and interferon on the growth rate of cells. *Virology* 1962; **17:** 324-8.

21. Pfeffer LM, Murphy JS, Tamm I. Interferon effects on the growth and division of human fibroblasts. *Exp Cell Res* 1979; **121:** 111-5.

22. Jezeršek B, Novaković S, Serša G, Auersperg M, Fleischmann WR Jr. Interactions of interferon and vinblastine on experimental tumor model melanoma B-16 *in vitro. In print (Anti-Cancer Drugs).*

23. Jezeršek B, Novaković S, Serša G, Čemažar M, Auersperg M, Fleischmann WR Jr. Interactions of interferon and vinblastine on experimental tumor model melanoma B-16 *in vivo. Radiol Oncol* 1993; **27:** 275-9.

24. Wandl UB, Niederle N, Kranzhoff M, Seeber S. Clonogenic assay is not predictive but reflects therapeutic efficacy of interferons in the treatment of chronic myelogenous leukemia. *Int J Cell Cloning* 1992; **10:** 292-8.

25. Kirkwood MJ. Studies of interferons in the therapy of melanoma. *Semin Oncol* 1991; **18** (Suppl 7): 83-90.

26. Schuller J *et al.* Pharmacokinetic aspects of interferon alfa-2b after intrahepatic or intraperitoneal administration. *Semin Oncol* 1992; **19** (Suppl 3): 98-104.