Anti-Cancer Drugs 1994, **5**, pp. 239–243

Interleukin-2 in neoadjuvant therapy potentiates inhibitory activity of 5-fluorouracil and interferon in experimental liver metastases

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Our previous studies showed that interferon (IFN) used in combination with 5-fluorouracil (5-FU) was effective in inhibiting colorectal tumor cell metastases to the liver in nude mice. Furthermore, IFN was also effective in neoadjuvant therapy and allowed the combination treatment (5-FU + IFN) to be delayed for 2–3 weeks following i.s. injections of tumor cells. In this study, we have examined the potential of interleukin-2 (IL-2) to substitute for IFN in neoadjuvant therapy. IL-2 was found to be equally effective, if not superior, to IFN as a neoadjuvant in inhibiting liver and lung metastases with 5-FU + IFN. Moreover, the effect of IL-2 was demonstrable even after 1 week, whereas IFN did not have an effect until 2 weeks of neoadjuvant dosing. These studies demonstrate IL-2 to be more effective than IFN as an immunomodulatory agent in combination with 5-FU + IFN for the inhibition of liver metastases in nude mice.

Key words: Chemotherapy, interleukin-2, interferon, LoVo, metastases, neoadjuvant.

Introduction

The use of immunomodulatory agents in cancer therapy has received much attention in recent years. Two of the most frequently studied agents are the cytokines interferon (IFN)-α and interleukin-2 (IL-2).[1] IFN-α has been used effectively as an anti-tumor agent in *in vivo* experiments in animal model systems, as well as in humans though restricted to certain cancers such as hairy cell leukemia and renal cell carcinoma.[2] IFN-α has shown anti-proliferative effects on tumor cells *in vitro*[3] and *in vivo*.[4] However, exactly how IFN-α exerts its anti-tumor action is not completely understood. A broad spectrum of properties has been reported for IFN-α. These include inhibition of tumor growth,[5,6] promotion of partial reversal of the malignant phenotype,[7] enhancement of surface molecule expression, e.g. α-2 microglobulin, Fc receptors, tumor antigens and histocompatibility antigens.[8–11] The immunomodulatory function of IFN-α has been described as an augmentation of lymphocyte cytotoxic responses.[12] Cytotoxic natural killer (NK) cells and antibody-dependent cell-mediated cytotoxic responses have been shown to be significantly augmented by IFNs. The immunomodulatory phenomena encompasses effects on the natural immunity of the host, and include the NK cells, the lymphokine activated killer cells (LAK), T cells and macrophages.[13–15]

IL-2 has also shown anti-tumor activity in animal experiments.[16,17] It appears to be essential for the proliferation of antigen stimulated T lymphocytes and cytotoxic T cells.[18] IL-2 has also been effective in promoting the growth of NK cells. When given in high doses to mice bearing tumors, IL-2 has mediated tumor regression by generating LAK cells and cytotoxic T lymphocytes.[19,20] There appears to be no evidence that IL-2 acts on non-lymphoid tumor cells. Thus it is currently presumed that IL-2 exerts its anti-tumor effects through an interaction with the immune system of the host.

Although a number of reports have described the effectiveness of IFN-α and IL-2 as single agents for the treatment of various cancers, it appears that the most useful application of these agents has been in combination,[21–23] and also when used with chemotherapeutic agents.[24,25] These cytokines have demonstrated an enhancement of the activity of cytotoxic drugs. For example, when IL-2 was used in combination with adriamycin and LAK cells on mice bearing advanced renal cell carcinoma, more long-term survivors were produced than by treatment with any of these agents alone or in other combinations.[26] Wadler and Schwartz[27] have re-

This work was supported in part by grants from the Pittsburgh National Bank Charitable Trust Fund and from Roche Laboratories.

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viewed an extensive literature dealing with the synergistic use of IFN as a modulating agent when used with cytotoxic agents in human malignancies.

We previously demonstrated that IFN-α is effective against colorectal tumor cell metastases to the liver in nude mice when used in combination with 5-fluorouracil (5-FU).[28] This treatment was initiated 3 days after tumor cells were injected into the spleen. In other studies from our laboratory, Lee *et al.*[29] demonstrated that IFN-α was also effective when used in neoadjuvant therapy *before* the initiation of chemotherapy and the removal of the tumor-bearing organ.

We now report a comparison of the *in vivo* antimetastatic effects of using IL-2 versus IFN-α in neoadjuvant therapy. When mice were treated with IL-2 for 1, 2 and 3 weeks after tumor cell injections, but before splenectomy and starting chemotherapy with 5-FU + IFN-α, the results showed that the effect of IL-2 was equal to IFN-α in inhibiting both liver and lung metastases at 2 and 3 weeks. At 1 week, the effect of IL-2 was superior to IFN-α against both liver and lung metastases. Thus, the effects of IL-2 can be expressed earlier and it appears to be a more potent immunomodulator than IFN-α.

Materials and methods

Athymic nude female mice were purchased from Harlan Sprague-Dawley (Indianapolis, IN). All mice were housed and maintained under specific pathogen-free conditions in the Nude Mouse Facility of the Mercy Cancer Center. Six week old mice were injected intrasplenically (i.s.) with tumor cells (1.5×10^6 cells in 0.050 ml) by the technique described by Kozlowski *et al.*[30] The use of the LoVo cell line has been described previously.[28,29]

Recombinant IFN-2α (Roferon A) and recombinant human rIL-2 were obtained as gifts from Roche Laboratories (Hoffman-La Roche, Nutley, NJ). Each vial of IFN-α contained 18×10^6 IU, with a specific activity of 2×10^8 IU/mg protein. Sterile water was used to reconstitute the contents of each vial. IFN-α was given s.c. at a dose of 3×10^5 units/injection in 0.2 ml. Each vial of rIL-2 containing 1×10^6 IU was reconstituted with 1.0 ml of sterile saline. Mice were injected i.p. with 5×10^4 units in 0.050 ml. 5-FU was given i.p. on the basis of 80 mg/kg.

Experiments were started on Fridays when mice were injected i.s. with LoVo cells while under methoxyflurane anesthesia. Splenectomies were performed on three groups, 1, 2 and 3 weeks later. IL-2 or IFN-α was given every other day (except weekends) during the interval between intrasplenic injection and splenectomy. Thus, there were two, five and eight injections of IL-2 or IFN-α in the 1, 2 and 3 week splenectomy groups of mice, respectively. Three days after splenectomy, all mice were started on the combined 5-FU + IFN schedule which has been described earlier.[28] Briefly, this consisted of 5-FU on Mondays and IFN daily. This schedule was repeated for 4 weeks. Mice were sacrificed 8 weeks after receiving the intrasplenic injections of tumor cells. Histological sections of livers and lungs were studied for the presence of metastases.

Results

We showed previously that a treatment schedule consisting of 5-FU (once a week) and IFN-α (daily) for four successive weeks following i.s. injection of LoVo cells resulted in significant inhibition of liver metastases.[28] We have now explored the use of neoadjuvant therapy which was combined with splenectomies and the regular 5-FU + IFN-α treatment. Splenectomies were performed 1, 2 and 3 weeks after i.s. injections. The 5-FU + IFN-α schedule started 3 days after splenectomy. During the interval between i.s. injection and splenectomy, IFN-α or IL-2 was given as neoadjuvant therapy.

The data in Table 1 shows a comparison of the results obtained when IFN-α and IL-2 were tested separately as neoadjuvants in combination with splenectomy and the combined 5-FU + IFN-α treatment. Both IFN-α and IL-2 are more effective at 2 and 3 weeks in inhibiting liver and lung metastases than in the control series (which had the same treatment except for the neoadjuvant drug). In fact, the effects of IFN-α and IL-2 parallel each other at the 2 and 3 weeks. However, a difference between IFN-α and IL-2 is noted for both liver and lung at 1 week, and this difference is significant at the $p < 0.05$ level. This is evident in Figure 1 which plots the percentage inhibition of metastases for both liver and lung at the three time points.

Discussion

This study was undertaken to examine the potential of IL-2 to be used as a neoadjuvant in place of IFN-α in a treatment regimen against hepatic metastases consisting of combined 5-FU + IFN-α. We had demonstrated previously that IFN-α was able to po-

Table 1. The effect of IFN-α and IL-2 in neoadjuvant therapy before splenectomy and treatment with combined 5-FU + IFN-α on liver and lung metastases

	Incidence of metastasis[a]	
	liver	lung
Controls		
Spl. 1 wk + 5-FU + IFN-α	16/24	10/24
Spl. 2 wk + 5-FU + IFN-α	16/24	12/24
Spl. 3 wk + 5-FU + IFN-α	18/22	18/22
IFN-α		
Pre-treat 1 wk IFN-α, Spl., +5-FU + IFN-α	20/22	18/22
Pre-treat 2 wk IFN-α, Spl., +5-FU + IFN-α	6/22	8/22
Pre-treat 3 wk IFN-α, Spl., +5-FU + IFN-α	6/26	4/26
IL-2		
Pre-treat 1 wk IL-2, Spl., +5-FU + IFN-α	10/18	8/18
Pre-treat 2 wk IL-2, Spl., +5-FU + IFN-α	6/20	8/20
Pre-treat 3 wk IL-2, Spl., +5-FU + IFN-α	4/16	4/16

[a]Incidence = no. of mice with metastasis/no. of mice injected.

tentiate the effects of 5-FU + IFN-α when used as a neoadjuvant before initiating therapy.[29] The data in Tables 1 and 2 and Figure 1 show (i) IL-2, like IFN-α, is able to potentiate the combination of 5- FU + IFN-α to inhibit liver and lung metastases at the 2 and 3 week levels, (ii) there is no inhibitory activity of IFN-α at 1 week in either liver or lung series, and (iii) IL-2 shows inhibitory activity at 1 week and the difference in effect between IL-2 and IFN-α is significant ($p < 0.05$). It appears that IL-2 is a more potent immunomodulatory agent than IFN-α since (i) the effect of IL-2 is evident at 1 week and not seen for IFN-α, and (ii) this is brought about by only two doses of IL-2, whereas IFN-α needs five doses to show an effect at the second week.

Table 2. Percent inhibition of liver and lung metastases by IFN-α and IL-2 in neoadjuvant therapy

	Liver (%)	Lung (%)
Controls		
Spl. 1 wk + 5-FU + IFN-α	33	58
Spl. 2 wk + 5-FU + IFN-α	33	50
Spl. 3 wk + 5-FU + IFN-α	18	18
IFN-α		
Pre-treat 1 wk IFN-α, Spl., +5-FU + IFN-α	9	18
Pre-treat 2 wk IFN-α, Spl., +5-FU + IFN-α	73	64
Pre-treat 3 wk IFN-α, Spl., +5-FU + IFN-α	77	85
IL-2		
Pre-treat 1 wk IL-2, Spl., +5-FU + IFN-α	44	56
Pre-treat 2 wk IL-2, Spl., +5-FU + IFN-α	70	60
Pre-treat 3 wk IL-2, Spl., +5-FU + IFN-α	75	75

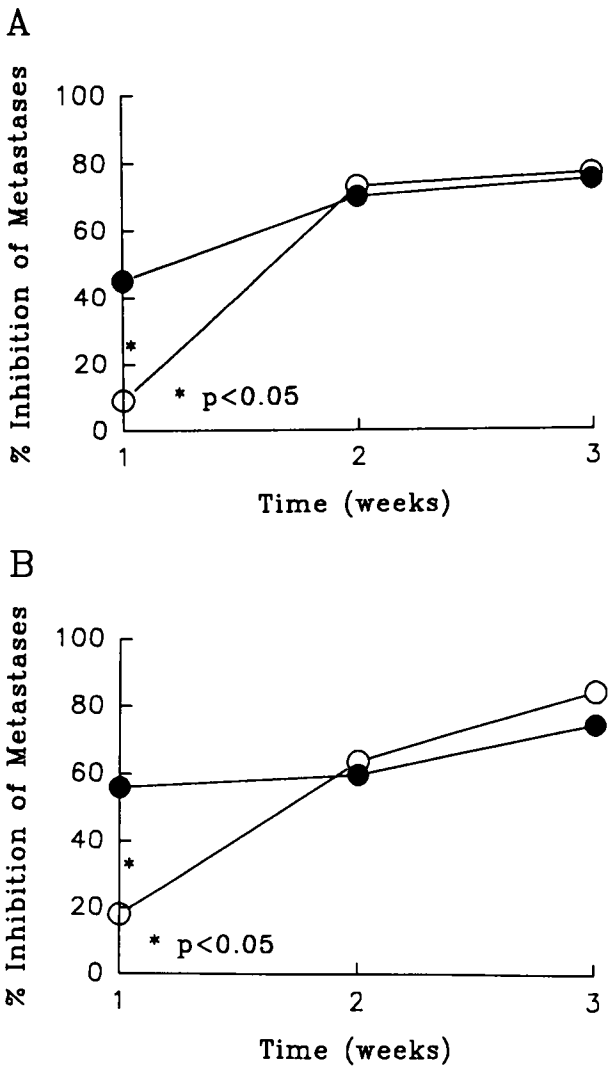

Figure 1. Percentage inhibition of (A) liver and (B) lung metastases by IL-2 (●) and IFN-α (○) when used as neoadjuvants for 1, 2 and 3 weeks prior to splenectomy, and combined treatment with 5-FU + IFN.

A variety of responses have been reported following the systemic administration of IL-2 to nude mice: the induction of specific T helper cells, cytotoxic cells and autoantibody production.[31–33] Rosenberg *et al.*[34] have shown that using only high doses of IL-2 in mice mediated the regression of established

lung and liver metastases and other subcutaneous tumor implants. In another study, they found that the effect of high dose IL-2 was absent in mice immunocompromised by 500 rad total body irradiation, or made T cell deficient by adult thymectomy and lethal body irradiation followed by reconstitution with T cell depleted bone marrow and spleen cells.[35] This suggested that the action of IL-2 was mediated by a component of the host, rather than exerting a direct effect on the tumor. Our experiments are not as complex, but nonetheless are similar in that T cell deficient mice were used. We have also delivered IL-2 via the i.p. route in low, sustained concentrations which have been noted to favor the generation of high levels of endogenous lymphocytes with LAK activity.[36] Further studies are needed to determine whether host-generated, cellular mediators of IL-2-induced inhibition of metastases are responsible for the effects described here.

Acknowledgments

The authors are grateful to the following individuals for skilled technical assistance which made this work possible: Jerry Glass, Marilyn Cost, Helen Fedorka, and Kurt Blanock. They also thank Albert Marrangoni, MD, Director of the Surgical Research Laboratory of the Department of Surgery, for support in carrying out this project.

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(Received 17 January 1994; accepted 24 January 1994)