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Interactions of interferon and vinblastine on experimental tumor model melanoma B-16 *in vivo*

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In the study, we investigated the in vivo *interaction of two antitumor agents, that have different sites and different mechanisms of action. Vinblastine (VLB) in combination with human recombinant interferon α A/D (rHuIFN-α A/D) and in combination with human leukocyte interferon α (HuLIFN-α) was tested on intraperitoneal (i.p.) melanoma B-16 tumor model. The effect of the combination was determined with follow-up of animals' survival and the interaction defined by means of Spector's formula. Only subadditive enhancement of interferon's (IFN's) antitumor activity was observed when rHuIFN-α A/D was combined with VLB and supraadditive, but not synergistic, interaction when HuLIFN-α was combined with VLB. Synergism between VLB and rHuIFN-α A/D on B-16 melanoma* in vitro, *that had been observed in our previous study, did not come true* in vivo.

Key words: melanoma, experimental-drug therapy; vinblastine; interferon alpha, recombinant;

Introduction

Chemotherapy and biotherapy are the two systemic modalities available for cancer treatment. However, because it is apparent that neither one nor the other are perfect treatments for cancer, the combination of cytotoxic drugs and cytokines offers a new approach to increase the therapeutic index in the treatment of neoplastic diseases.[1,2]

Interferons (IFNs) are a complex group of cytokines with antiviral, antibacterial, antitumor and immunomodulatory activities.[3,4] They exert antiproliferative effect on tumor cells, while IFNs β and γ also have a direct cytotoxic activity.[5] Antitumor activity of VLB is a consequence of its binding to microtubular proteins of the mitotic spindle, which causes metaphase arrest of cells in mitosis.[6,7] VLB is, in higher concentrations, also directly cytotoxic for interphase cells.[8]

While *in vitro* studies have demonstrated both direct cytotoxic and cytokinetic effects of IFNs, a more interesting role derives from their ability to sinergistically potentiate the wide variety of cytotoxic agents against multiple human and rodent tumors, both *in vitro* and in animal models.[9] The broad spectrum of cytotoxic drugs whose activity can be enhanced by cytokines argues for multiple levels of drug interaction *in*

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vitro: alteration of cellular drug uptake, modulation of drug target enzymes, and changes in metabolism or disposition of a drug. *In vivo* interaction between cytokines and cytotoxic drugs involves an additional layer of complexity because of the effects of cytokines on the host immune system and on drug-metabolising enzymes.[2]

The ability of IFNs to directly modulate the biochemical effects of cytotoxic agents independent of immunomediated or host-protective effects has been evaluated in a variety of *in vitro* systems.[9] Since synergistic cytotoxicity has been observed *in vitro* for IFN-α combination with VLB on BG-1 human ovarian carcinoma line,[10] on RPMI 8226 human myeloma line, on MCF-7 human breast carcinoma line, on WiDr human colon carcinoma line[11] and on murine B-16 melanoma line,[12] we wanted to define the interaction of VLB with rHuIFN-α A/D or HuLIFN-α *in vivo* on i.p. B-16 melanoma tumor model.

Materials and methods

Reagents

Recombinant HuIFN-α A/D was provided by Hoffmann-LaRoche (Nutley, New Jersey) and HuLIFN-α by Immunological Institute (Zagreb, Croatia). Both were diluted with phosphate buffered saline (PBS).

Vinblastine sulfate (Lymphomed, Deerfield, Illinois) was used in combination with rHuIFN-α A/D and Velbe (Lilly, Firenze, Italy) with HuLIFN-α. Both were diluted with PBS.

Animals

Six to eight weeks old pathogen-free female C57Bl/6 mice were purchased from Jackson Laboratories, Bar Harbor, USA. Animals were maintained in a pathogen-free state in animal rooms with alternating cycles of 12 h light and 12 h darkness. Each experimental group consisted of 10 to 11 mice. These animals were used for experiments with rHuIFN-α A/D and Vinblastine sulfate.

Female C57Bl/6 were purchased from Rudjer Bošković Institute, Zagreb, Croatia. Animals were maintained at a natural day/night cycle in a standard animal colony. Eight to ten weeks old mice in good condition without any signs of fungal or other infections were used in the experiments. Each experimental group consisted of ten mice. These animals were used for experiments with HuLIFN-α and Velbe.

Tumor cells

Murine B-16 melanoma cells (clone F1, American Type Culture Collection, Rockville, Maryland) were grown in Eagle's Minimal Essential Medium supplemented with 10% fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml) and gentamycin (11 μg/ml). These cells were used for experiments with rHuIFN-α A/D and Vinblastine sulfate.

Murine B-16 melanoma cells (clone B6, Rudjer Bošković Institute, Zagreb, Croatia) were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, gentamycin (500 μg/ml) and 7,5% sodium bicarbonate (27 ml/l). These cells were used for experiments with HuLIFN-α and Velbe.

Experimental procedure

Mice were i.p. injected with 10^6 B-16 melanoma (clone F1 or B6) cells on day 0 and randomly divided into four groups. All treatment was intraperitoneal and was administered as follows:

– control group - PBS for five consecutive days, starting day 1

– vinblastine group - Vinblastine sulfate or Velbe (30 μg per animal) on day 4 only

– interferon group - rHuIFN-α A/D (1×10^5 I.U. per animal) or HuLIFN-α (5×10^5 I.U. per animal) for five consecutive days, starting day 1

– combination group - Vinblastine sulfate or Velbe (30 μg per animal) on day 4 only + rHuIFN-α A/D (1×10^5 I.U. per animal) or HuLIFN-α (5×10^5 I.U. per animal) for five consecutive days, starting day 1.

The mice were monitored for the day of death and the average day of death was determined.

Statistical analysis

The Mantel - Cox test (BMDP Statistical Software, Los Angeles, California) was employed for comparison of the animals' survival and Spector's formula[13] to define the interaction of rHuIFN-α A/D or HuLIFN-α with VLB.

Results

Vinblastine sulfate and rHuIFN-α A/D as single agents or in combination were tested for their effect on survival of animals with i.p. B-16 melanoma (F1). Intraperitoneal application of 30 μg of Vinblastine sulfate on day 4 had a moderate ($p = 0{,}054$) antitumor effect, while treatment with 1 x 10^5 I.U. of rHuIFN-α A/D for 5 consecutive days showed a more pronounced statistically significant ($p<0{,}001$) effect on survival of mice with i.p. B-16 melanoma. The combination of both agents had a statistically significant ($p<0{,}001$) antitumor effect, but there was no significant difference ($p = 0{,}497$) in survival between the "interferon group" and the "combination group" of animals (Figure 1). According to Spector's formula the interaction of rHuIFN-α A/D with VLB was merely subadditive and the antitumor effect of the combination was 90% of the one expected on the basis of their separate activities.

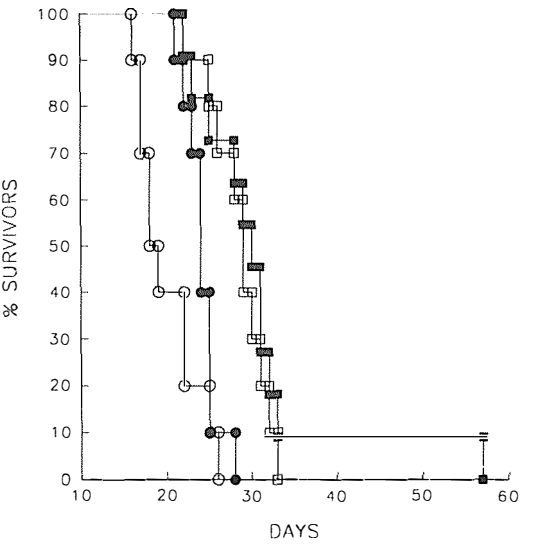

Figure 1. Survival of mice with i.p. B-16 melanoma (clone F1) treated with Vinblastine sulfate (●), rHuIFN-α A/D (□) or combination of both agents (■); control (○). The antitumor effect of the combination was merely subadditive in comparison to the one expected on the basis of separate activities of VLB or IFN.

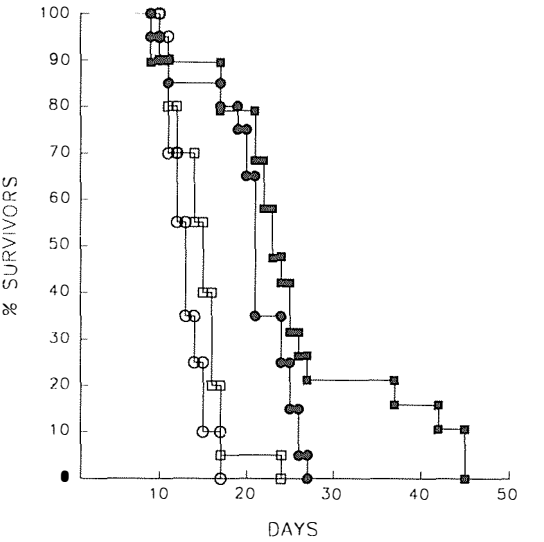

Figure 2. Survival of mice with i.p. B-16 melanoma (clone B6) treated with Velbe (●), HuLIFN-α (□) or combination of both agents (■); control (○). The effect of the combination on survival of the animals was supraadditive in comparison to the one expected on the basis of separate activities of VLB or IFN.

Survival of animals with i.p. B-16 melanoma (B6) treated with HuLIFN-α or Velbe alone or in combination is presented in Figure 2. Treatment with 5 x 10^5 I.U. of HuLIFN-α for five consecutive days had a moderate ($p = 0{,}059$) antitumor effect, while i.p. application of 30 μg of Velbe significantly ($p<0{,}001$) prolonged survival of the animals. The combination of both agents had a statistically significant ($p<0{,}001$) antitumor effect, but there was no significant difference ($p = 0{,}058$) in survival between the "vinblastine group" and the "combination group" of animals. The interaction of HuLIFN-α with VLB was supraadditive and the antitu-

mor effect of the combination was 115% of the one expected on the basis of their separate activities.

Discussion

The results of *in vivo* studies testing the combination of IFNs with VLB are controversial. Sidkey *et al.* report that murine IFN α/β increased survival in mice with P388 leukemia cells after treatment with VLB.[14] Harrison *et al.* on the other hand found no positive interaction when murine IFN α/β was combined with VLB on s.c. Meth A sarcoma tumor model and when recombinant murine IFN-γ was combined with VLB on s.c. Meth A sarcoma and s.c. B-16 melanoma.[15] Also Mitchell has pointed out that although type I IFN has been found to potentiate chemotherapy in cultured cells, "there is very little substantiation *in vivo*".[16]

Our results clearly demonstrate that *in vitro* synergism between rHuIFN-α A/D and VLB observed on F1 clone of B-16 melanoma cells[12] did not come true *in vivo*. However, there is an interesting difference between the antitumor activity of combination of rHuIFN-α A/D with VLB and combination of HuLIFN-α with VLB. Even though HuLIFN-α alone has only moderate antitumor activity, the interaction with VLB was supraadditive, in comparison with rHuIFN-α A/D that has significant antitumor activity, but demonstrated only subadditive interaction with VLB. In part this difference could be explained with the fact that different clones of B-16 melanoma were used in the experiments, but Sklarin *et al.* report that in most of the cases where potentiation was observed, human IFN-α alone had only weak antitumor activity; however, IFN-α seemed to be most effective in combination with drugs that alone possessed substantial activity against the specific tumor.[17]

The question of the mechanism of interaction between IFNs and VLB still cannot be resolved and it seems likely that multiple factors may be contributing to success or failure in these preclinical models. The interactions observed are not solely the consequence of the combined effect of two cytoreductive agents, since the enhanced activity of the drug-interferon combination was observed even in instances where IFNs alone lacked activity, and IFNs also failed to potentiate the activity of other efficacious drugs.[9] There is also a complex relationship between the timing of interferon (IFN) with a cytotoxic agent, the doses used, and the efficacy of the regimen. In combination with cytotoxic drug, sequence and duration of exposure to IFN may play as significant a role as dose and dose intensity, and the maximum tolerated dose of IFN may not be the most biologically effective dose. Up till now, the lack of understanding of the biochemical interaction of these agents has prohibited a rational approach to design of schedule and sequence that allow translation of the positive *in vitro* data into effective preclinical treatment regimens.

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