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

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In this study, we tried to define *in vitro* interactions 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 murine recombinant interferon-γ (rMuIFN-γ) was studied. The effect of the combination was determined with cell growth kinetics assay on B-16 melanoma and the interaction defined by means of Spector's formula. Both the combination of rHuIFN-α A/D with VLB and the combination of rMuIFN-γ with VLB synergistically inhibited cell growth *in vitro*. There was a positive biochemical modulation between the two drugs, but it is still unknown whether it occurred at the level of uptake into the cell, metabolism within the cell or egress from the cell.

Key words: B-16 melanoma, cell culture, human recombinant IFN-α A/D, murine recombinant IFN-γ, synergy, vinblastine.

Introduction

Interferons (IFNs) are a complex group of cytokines with antiviral, antibacterial, antitumor and immunomodulatory activities.[1,2] They exert an antiproliferative effect on tumor cells, while IFN-β and -γ also have a direct cytotoxic activity.[3] Antitumor activity of vinblastine (VLB) is a consequence of its binding to microtubular proteins of the mitotic spindle, which causes metaphase arrest of cells in mitosis.[4,5] VLB is, in higher concentrations, also directly cytotoxic to interphase cells.[6]

IFN-α, -β, and -γ have been studied *in vitro* in combination with various antineoplastic drugs against established cell lines and fresh explanted human tumors. Potentiation of the antiproliferative effect of IFN has been reported for many such combinations, using a number of different methodologies to assess for potential synergism.[7–11] However, there is as yet no consensus on optimal strategies for combining IFNs with antineoplastic drugs. Since identification of synergistic antitumor effects of combination therapy is necessary at both preclinical and clinical levels, methodological approaches for documentation of synergism have become increasingly important.[7,8]

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Materials and methods

Tumor cells

B-16 (clone F_1) melanoma cells (American Type Culture Collection, Rockville, MD) were grown in Eagle's minimal essential medium (EMEM) supplemented with 10% fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml) in gentamycin (11 μg/ml) at 37°C in a humidified CO_2 incubator. They were routinely passaged every 2–3 days.

Log-phase B-16 melanoma cells were trypsinized, centrifuged, resuspended in growth medium and plated on 35 mm gridded tissue culture dishes at a concentration of 2×10^3 cells/dishes. After an incubation period of 24 h, the growth medium was removed and 1 ml of growth medium alone or growth medium containing IFN or VLB was added back to each plate. Shortly after this change of medium, the day 0 cell counts were made. Areas of grids containing 5–10 cells/area were located and the cell counts for 20 such areas were recorded for each growth condition. Cell growth in the test areas was monitored by counting the cells in each grid area at 24 h intervals for 3 days. The growth of the

cells in each grid area for each test condition was determined for days 1–3 of the experiment by dividing the number of cells in the grid area on the indicated day by the number there on day 0. The 20 values for each day were averaged.[12]

Reagents

Hybrid recombinant human IFN-α (rHuIFN-α) A/D ($10^{7.81}$ units/mg of protein) was provided by Hoffman-LaRoche (Nutley, NJ) and recombinant murine IFN-γ (rMuIFN-γ) ($10^{7.0}$ units/mg of protein) by Schering (Bloomfield, NJ). Both were diluted to appropriate concentrations in EMEM.

Vinblastine sulfate (Lymphomed, Deerfield, IL) was used and the dilutions were made with growth medium.

Spector's formula

Spector's formula was used to define the interaction of IFN and VLB (antagonism, additivity or synergism).[13] According to the formula, we determined the value Q and standard error for all test conditions together. Comparison of Q with standard error defined whether the interaction was synergistic, additive or antagonistic.

Results

Antitumor effect of rHuIFN-α A/D

Three different concentrations (100, 1000 and 10 000 IU/ml) of rHuIFN-α A/D were tested for their effect on growth of B-16 melanoma cells *in vitro* (Figure 1). The exposure to rHuIFN-α A/D was continuous. The antiproliferative effect of lower two concentrations of rHuIFN-α A/D was minimal, while the highest concentration moderately inhibited tumor cell growth.

Antitumor effect of rMuIFN-α

The antitumor effect of three different concentrations (10, 30 and 100 IU/ml) of rMuIFN-γ was determined on B-16 melanoma cells *in vitro* (Figure 2). The exposure to rMuIFN-γ was continuous. The antiproliferative effect of rMuIFN-γ was more pronounced than the one of rHuIFN-α A/D, but was still moderate in degree. The higher two

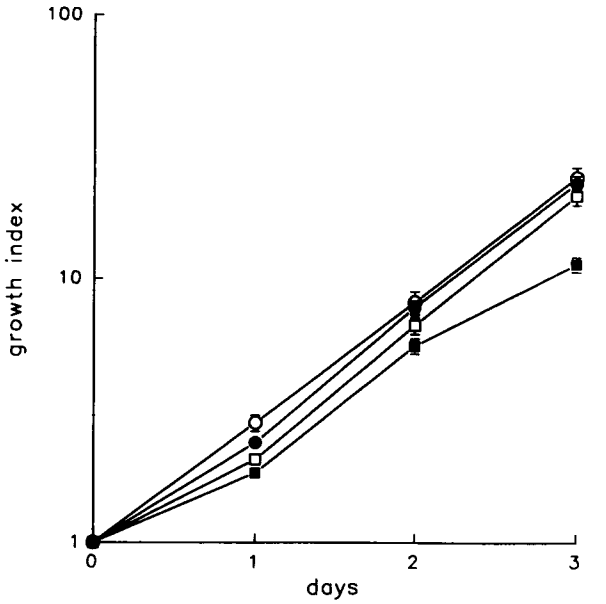

Figure 1. The effect of three different concentrations (●, 100 IU/ml; □, 1000 IU/ml; ■, 10 000 IU/ml; ○, control) of rHuIFN-α A/D on growth of B-16 melanoma cells *in vitro*. The exposure to rHuIFN-α A/D was continuous.

concentrations had nearly the same effect. No cytotoxic effect was shown for the concentrations used in this experiment.

Antitumor effect of VLB

Three different concentrations (3, 30 and 300 ng/ml) of VLB were tested for their antitumor effect on

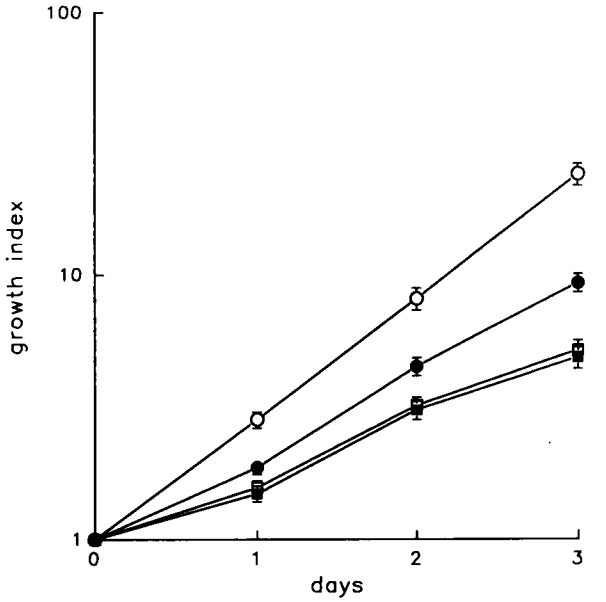

Figure 2. The antitumor effect of three different concentrations (●, 10 IU/ml; □, 30 IU/ml; ■, 100 IU/ml; ○, control) of rMuIFN-γ on B-16 melanoma cells *in vitro*. The exposure to rMuIFN-γ was continuous.

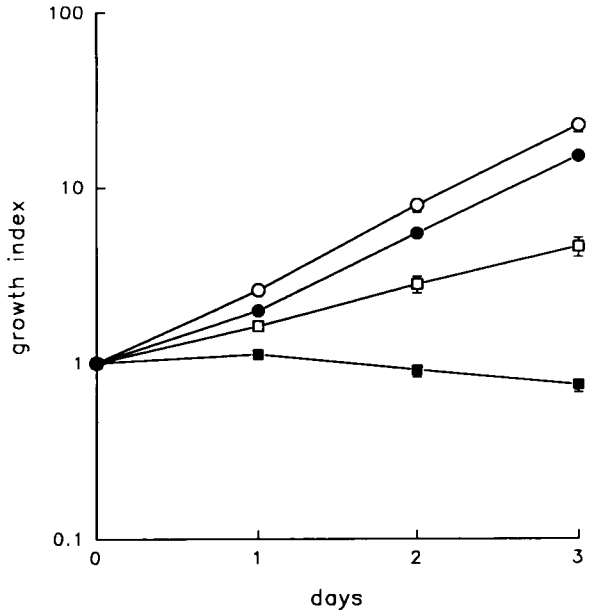

Figure 3. The antitumor activity of three different concentrations (●, 3 ng/ml; □, 30 ng/ml; ■, 300 ng/ml; ○, control) of VLB on B-16 melanoma *in vitro*. The exposure to VLB was 6 h on day 0.

B-16 melanoma cells *in vitro* (Figure 3). The exposure to VLB was 6 h on day 0. The antiproliferative activity of VLB depended on the concentration of the drug. The highest concentration of VLB showed cytotoxic activity.

Antitumor effect of combined IFN and VLB treatment

The antitumor activity of the combination of VLB (300 ng/ml) with rHuIFN-α A/D (10 000 IU/ml) (Figure 4A) or with rMuIFN-γ (10 IU/ml) (Figure 4B) was studied on B-16 melanoma cells *in vitro*. Exposure to either of the IFNs was continuous, while to VLB it was 1 h on day 1. According to Spector's formula both combinations synergistically inhibited tumor cell growth. Synergism was observed as greater than additive enhancement of IFN's antitumor activity when rHuIFN-α A/D or rMuIFN-γ were combined with VLB. Enhancement levels observed were 1.4- to 1.7-fold greater than expected on the basis of their separate activities.

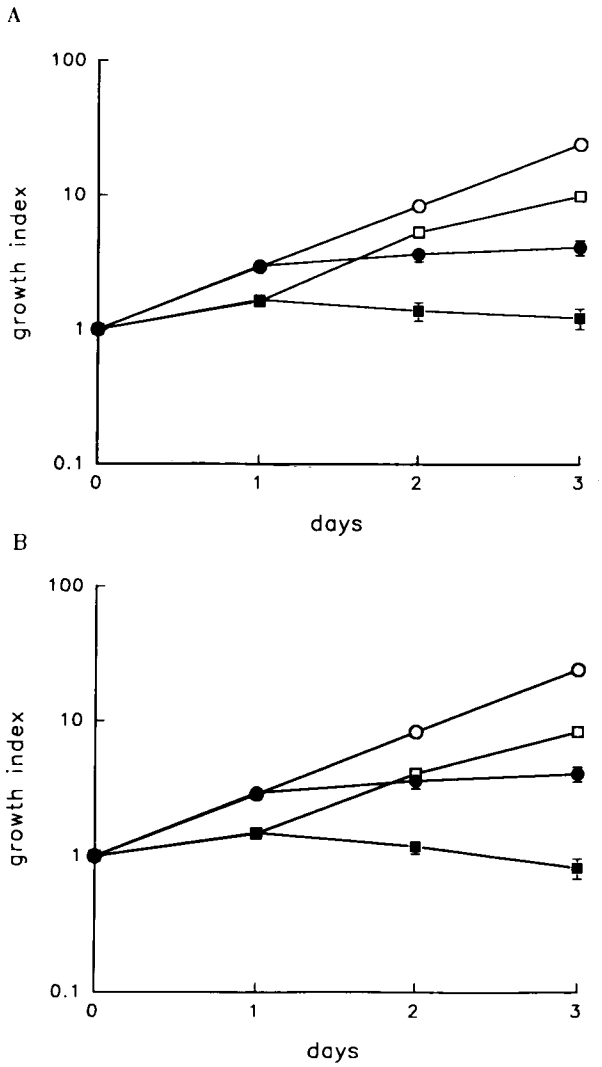

Figure 4. (A) The effect of combination of VLB with rHuIFN-α A/D on growth of B-16 melanoma *in vitro*. The exposure to rHuIFN-α A/D was continuous, while to VLB it was 1 h on day 1. ○, Control; ●, VLB (300 ng/ml); □, rHuIFN-α A/D (10 000 IU/ml); ■, VLB (300 ng/ml) + rHuIF-N-α A/D (10 000 IU/ml). (B) The antitumor activity of combination of VLB with rMuIFN-γ on B-16 melanoma cells *in vitro*. The exposure to rMuIFN-γ was continuous, while to VLB it was 1 h on day 1. ○, Control; ●, VLB (300 ng/ml); □, rMuIFN-γ (10 IU/ml); ■, VLB (300 ng/ml) + rMuIFN-γ (10 IU/ml).

Discussion

The interaction between IFNs and cytotoxic agents *in vitro* is complex and depends not only on the choice of cytotoxic agent but also on the concentrations, ratios, duration and sequence of exposure to the two drugs. Data suggest that some combinations are not merely additive but rather that IFNs may biochemically modulate the metabolism of the cytotoxic agent resulting in synergistic antineoplastic activity.[8] The broad spectrum of cytotoxic drugs whose activity can be enhanced by cytokines argues for multiple levels of drug interaction *in vitro*: alteration in the cellular drug

uptake, modulation of drug target enzymes and changes in metabolism or disposition of a drug.[14]

Our results clearly demonstrate that the combinations of rHuIFN-α A/D or rMuIFN-γ with VLB have direct cytotoxic interactions in B-16 melanoma cells. Little is known about the nature of these interactions, but it is likely that there is a modulation of the antiproliferative activity of either VLB or the IFN used (or possibly both). IFNs potentiated the actions of a range of cytotoxic agents with multiple and varying mechanisms. This suggests that no single interaction is operative but rather that the synergy observed results from a combination of two broadly defined areas of interaction: an enhancement of the activity of IFN or the anticancer agent by a specific biochemical interaction, relevant only to a particular combination; or potentiation due to specific actions on cell growth, replication, differentiation or the response to cellular damage, which potentiate the activity of many antiproliferative agents.[8]

Kuebler *et al.* demonstrated that recombinant IFN-β enhances the uptake of VLB into non-resistant renal carcinoma cells, while the egress of the drug remains unaltered.[7] This membrane–IFN interaction may produce an increase in the effective VLB concentration within the cell, resulting in greater cell growth inhibition. It is unlikely, however, that membrane alterations are responsible for all of the enhanced effect of the combination.

The effects of IFN on the pharmacokinetics of chemotherapeutic agents, on the metabolism of chemotherapeutic agents and on interactions of the chemotherapeutic agents with the cell genome still remain to be studied in order to enlighten the mechanisms of interaction.

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