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Interaction between human lymphoblastoid interferon and chemotherapeutic agents in vitro.

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Abstract

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KEYWORDS: human lymphoblastoid interferon, in vitro chemotherapy, interaction of anticancer agents

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INTERACTION BETWEEN HUMAN LYMPHOBLASTOID INTERFERON AND CHEMOTHERAPEUTIC AGENTS *IN VITRO*

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Abstract. The combined effect of human lymphoblastoid interferon (HLBI) and anticancer agents on the growth of MOLT-4 was studied *in vitro.* The interferon showed a strikingly synergistic interaction in combination with aclarubicin, cytosine arabinoside or prednisolone. It was moderately synergistic in combination with adriamycin or 5-fluorouracil and tended to show additive effects with daunorubicin or vincristine. *In vitro* studies of combination chemotherapy with interferon and anticancer agnets should yield valuable information as to the best combination for man.

Key words : human lymphoblastoid interferon, *in vitro* chemotherapy, interaction of anticancer agents.

The development of a technique for the mass production and the purifIcation of human interferon has made clinical trials in man possible. Recent cilnical trials of interferon conducted in our country have shown activity in the treatment of renal cell carcinoma, multiple myeloma and malignant lymphoma (1-3).

It is well known that the effect of anticancer agents in combination is superior to that of individual agents. Therefore, interferon-anticancer agent chemotherapy may be expected to be effective in the clinical management of neoplastic diseases.

In vitro chemotherapy using human tumor cells has been widely applied not only to the investigation of the action mechanism, but also to the evaluation of the optimal agents for man. In this paper the combined effect of interferon and established chemotherapeutic agents was assessed in order to gain some information on combined interferon-anticancer agent therapy in man.

MATERIALS AND METHODS

Tumor cells. A human acute lymphocytic leukemia (ALL) cell line, MOLT-4, with T-cell characteristics was maintained in our laboratory. The cells were maintained in suspension culture flasks containing RPMI Medium 1640 with 10 % fetal calf serum and fed with fresh medium 3 times a week. Cells in stock culture were counted daily and used when they were

in an exponential phase.

Drugs. The interferon used in this experiment was human lymphoblastoid interferon (HLBI) prepared from cells of the human B-lymphoblastoid line (4), Namalwa, stimulated with Sendai virus, which was kindly supplied by Sumitomo Kasei Co. (Osaka). Anticancer agents used in combination with the interferon included aclarubicin (ACR), daunorubicin (DNR), adriamycin (ADM), cytosine arabinoside (ara-C), 5-fluorouracil (5-FU), vincristine (VCR) and prednisolone (Pred), and they were obtained commercially. Drugs were diluted to the desired concentrations in physiological saline solution, and they were prepared freshly for each experiment.

Determination of the combined effect in vitro. The combined effect *in vitro* was determined according to Elion's method (5). Namely, the concentration of interferon and each drug alone required to achieve 70 % inhibition of the growth of MOLT-4 was determined from dose-response curves on day 4. When the fraction of these concentrations which in combination produced 70 % inhibition were plotted to a straight line connecting the two points 1.0 to 1.0, the drug combination had an additive interaction. Curves to the left of this line indicated a synergistic interaction, curves to the right, an antagonistic interaction. Growth inhibition was assessed by the trypan blue dye exclusion method. All experiments were carried out in triplicate and repeated at least twice.

RESULTS AND DISCUSSION

It has been shown that interferon can be effectively added to some conventional anticancer agents in the treatment of leukemia in mice. Gresser observed prolonged survival in AKR lymphoma mice treated with the combination of interferon and cyclophosphamide (CYC) (6). Chirigos also recognized a curative effect with the combination of interferon and bischlorethyl nitrosourea (BCNU) in mice bearing LSTRA leukemia (7). A previous report from our laboratory showed that a combination of interferon and CYC inhibited the growth of BALL-1 (a human B-ALL cell line) transplanted in newborn hamsters immunosuppressed with rabbit anti-hamster lymphocyte serum more than either agent alone (8). The combined use of interferon and L-asparaginase was also more inhibitory to TALL-1 (a human T-ALL cell line) than either agent alone *in vitro.* Recently, Slater found enhanced survival of L 1210 leukemic mice treated with the combination of interferon and methotrexate (MTX) (9).

Our study showed a striking synergistic interaction between interferon and ACR, ara-C and Pred (Figs. 1, 2). The combination of interferon with ADM and 5-FU was moderately synergistic, and interferon tended to show additive effects in combination with DNR or VCR *in vitro.* The synthesis of DNA and protein was found to be reduced in interferon-treated cells (10), which was thought to be one of the possible causes of the inhibition of cell growth. Interferon was also shown to suppress the transition of cells from the G_1 phase to the S phase (11). Whether or not these biological changes induced by interferon directly participate in the enhancement of anticancer agents is not yet clear. Because there is nothing in common among the anticancer agents showing a synergistic interaction with interferon, it is probable that the interactions between interferon and the different

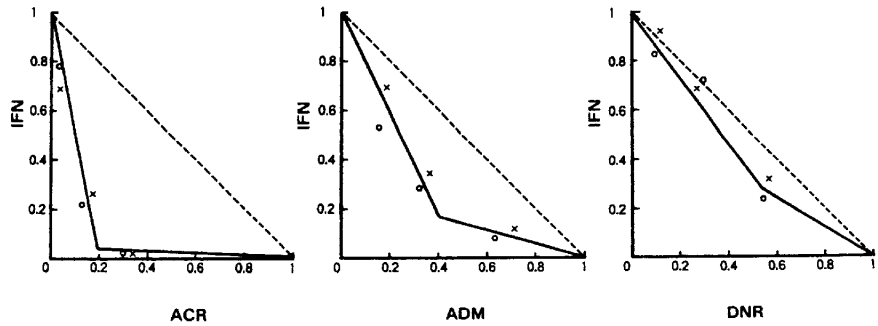

Fig. 1. Effects of Combination of Interferon with Anticancer Agents on the Growth of MOLT-4 *in vitro*

The concentration of each drug alone required for 70 % inhibition of growth is determined from dose response curves. The fractions of these concentrations which in combination produce 70 % inhibition are plotted to determine the type of interaction. If the plotted data resulted in a straight line connecting the two points 1.0 to 1.0, the drug combination has resulted in an additive interaction. Curves to the left of this line indicate a synergistic interction, and curves to the right, an antagonistic interaction. IFN: Human lyphoblastoid interferon (HLBI) x: Experiment 1 o: Experiment 2

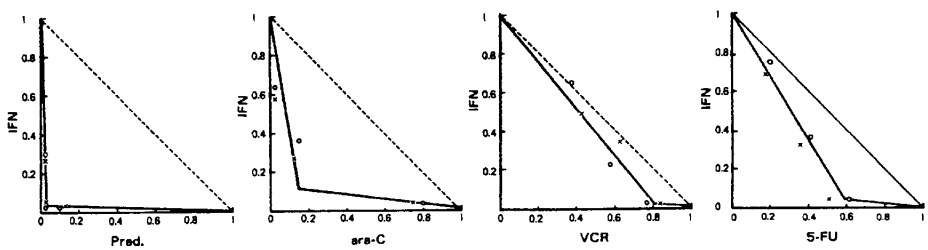

Fig. 2. Effects of Combination of Interferon with Anticancer Agents on the Growth of MOLT-4 *in vitro*

The concentration of each brug alone required for 70 % inhibition of growth is determined from dose response curves. The fractions of these concentrations which in combination produce 70 % inhibition are plotted to determine the type of interaction. If the plotted data resulted in a straight line connecting the two points 1.0 to 1.0, the drug combination has resulted in an additive interaction. Curves to the left of this line indicate a synergistic interaction, and curves to the right, an antagonistic interaction. IFN: Human lymphoblastoid interferon (HLBI) x: Experiment 1 o: Experiment 2

anticancer agents are unique. Since ACR, ADM, ara-C and Pred have already been found to be active in the treatment of hematological neoplasms such as leukemia, multiple myeloma and malignant lymphoma, combination chemotherapy with these agents and interferon may be expected to be useful in the clinical management of these diseases. Combined interferon-anticancer agent therapy has started in our country. *In vitro* studies on the interaction of interferon and conventional anticancer agents should yield useful information as to which drug are optimal for man.

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