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Therapeutic Concentrations of Antineoplastic Agents Diminish Interferon Yields (41483)

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Abstract. Interferon production was studied in the presence of therapeutic concentrations of five antineoplastic agents. None of the drugs studied grossly altered the production of interferon by fibroblasts. Virus-induced mononuclear cells, in contrast, produced significantly less interferon after prior exposure to vincristine and produced no detectable interferon after prior exposure to 6-MP. These studies establish that interferon yields *in vitro* may be diminished by therapeutic concentrations of antineoplastic agents, and necessitate *in vivo* studies to determine if this alteration in interferon production can help explain the increased severity of certain viral infections in patients with neoplastic diseases.

Patients with certain forms of malignant disease are known to be at increased risk for serious viral infections (1). Because of this fact, we have questioned whether interferon production may be diminished in malignant disease particularly by the chemotherapeutic agents used to treat neoplastic processes.

As a preliminary to investigations *in vivo*, we have studied the effects of various antineoplastic agents on the ability of human cells to produce interferon *in vitro*.

This report reviews our experience with the production of interferon in the presence of five antineoplastic agents. We have found that therapeutic concentrations of these agents can influence interferon production in human cells, especially mononuclear cells. We suggest *in vivo* studies be done to determine if this phenomenon occurs in cancer patients receiving chemotherapy.

Materials and Methods. *Cells. Fibroblasts.* Human foreskin fibroblasts (FF cells) originally prepared in our own laboratory were used for these experiments. Fibroblasts were grown to confluence in 25-cm^2 plastic screw-cap flasks prior to interferon induction.

Mononuclear leukocytes. Human leukocytes were obtained from the American Red Cross as the platelet-rich fraction of human blood. These preparations were tested within 24 hr of procurement. Upon receipt in the laboratory, the cell suspension was subjected to centrifugation on Ficoll–Hypaque for 45 min at 1000 rpm. The mononuclear cell layer was then harvested, washed three times with phosphate-buffered saline (PBS), and adjusted to a concentration of 1×10^7 cells/ml in Newman Tytell medium containing 10% bovine fetal serum (BFS).

Antineoplastic agents. Antineoplastic agents were obtained from the following sources: 5-fluorouracil from Roche Laboratories, Nutley, New Jersey; adriamycin from Farmitalia, SPS, Italy; vincristine from Eli Lilly, Indianapolis, Indiana; 6-mercaptopurine (6MP) from Burroughs Wellcome, Research Triangle Park, North Carolina; and methylprednisolone from Upjohn Company, Kalamazoo, Michigan.

All solutions were freshly prepared immediately prior to use. The concentrations employed in these experiments were chosen to approximate those present in the serum immediately following administration to humans (2–5).

Inducing agents. Complexed polyinosinic–polycydidylic acids (I:C) were obtained either in liquid or powdered form and were diluted just prior to use in phosphate-

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buffered saline (PBS) containing calcium and magnesium.

Diethylaminoethyl dextran (DEAE-D) was dissolved in water, in a concentrated form, and frozen at −20°. Immediately prior to use, an aliquot of DEAE-D was added to the I:C solution so that the final concentration of the inducing solution was 10 μg/ml of I:C and 100 μg/ml of DEAE-D.

Newcastle disease virus (NDV) was grown in eggs using a laboratory strain that had been serially passed through many generations.

Induction of interferon. Preliminary experiments demonstrated that the various concentrations of drugs employed were not toxic to the fibroblast monolayers (as evidenced by lack of morphologic changes) and would not induce interferon.

FF cells were grown to confluence in screw-cap flasks. At the initiation of the experiment three flasks of fibroblasts were exposed to L-15 media containing 2% bovine fetal serum, 1 mg/ml dextrose, 30 μg/ml glutamine, 90 μg/ml arginine, 150 u/ml penicillin, 250 μg/ml of streptomycin, and an appropriate concentration of the antineoplastic agent to be tested. Control flasks and simultaneous exposure flasks were treated with this same media without the antineoplastic agent during this 24-hr period. After the preliminary exposure, I:C induction was accomplished by adding 2 ml of the inducing solution containing the individual antineoplastic agents in the concentrations noted to both pretreatment and simultaneous exposure flasks. Control flasks were provided with 2 ml of the inducing solution without the antineoplastic agents.

All flasks were exposed to the inducing solutions for 3 hr and then washed with PBS. Subsequently, 2 ml of L-15 media supplemented as described above was added. Individual antineoplastic agents in the appropriate concentrations were added to the media of the test flasks (both pretreated and simultaneously treated), but control flasks contained the same media without such agents. The flasks were incubated overnight and the media was then harvested. All solutions were dialyzed for 24 hr against 100 vol of PBS to remove the antineoplastic agent, harvested, and frozen at −80° until assayed. Control and test inductions were performed simultaneously under identical circumstances save for the presence of the drug in the test solutions. All inductions were performed in triplicate.

In the case of induction with virus, duplicate flasks were prior treated as described above. At the appropriate time, the prior-treated flasks and unexposed flasks were suctioned and exposed to NDV in a concentration of 100 EID_{50} per cell for 1 hr. At the conclusion of this exposure, the two prior-treated flasks as well as the simultaneous exposure flasks were covered with 2 ml of the supplemented L-15 containing the antineoplastic agents. Duplicate control flasks, which had been exposed to virus but not to the test drugs, were covered with a similar quantity of supplemented L-15 without any antineoplastic drug.

Incubation was then carried out for 24 hr after which the media was harvested and dialyzed against 100 vol of 0.1 *M* citric acid for 4 days. The dialysis bath was then changed to PBS for 24 hr. Subsequently, the solutions were harvested and ultracentrifuged at 25,000 rpm for 90 min. Supernatants were harvested and stored at −80° until assayed.

Induction of interferon in mononuclear leukocytes was accomplished as follows: cells were adjusted to a concentration of 10^7 cells/ml in Newman Tytell media supplemented with 10% BFS, 250 u/ml of penicillin, and 150 μg/ml of streptomycin. Tubes used for the pretreatment experiments were exposed to media containing the appropriate concentration of antineoplastic agent for 24 hr at 37° in a 5% CO_2 incubator. Other tubes were simultaneously incubated in identical media save for the absence of the drugs.

Freshly prepared media was then added to all cells: the pretreatment and simultaneous exposure tubes receiving media containing the appropriate drug and the control tubes receiving the media without the drugs. NDV in a concentration of 100 EID_{50}/cell was added to each tube and incubation carried out for 24 hr in an incubator containing 5% CO_2. At the conclu-

sion of the incubation period the cells were centrifuged, the supernatant harvested, and the media dialyzed against citric acid for 4 days and PBS for 1 day. The media was then harvested, centrifuged for 90 min at 25,000 rpm, and frozen at −80° for later assay.

Viability counts were performed by the trypan blue exclusion method prior to discarding all cells. For all experiments at least 80% of the cells were viable at the *conclusion* of the experiment and the difference in viability between test and control tubes was always less than 10%.

Interferon assays. Interferon was assayed using a microtiter method which employed vesicular stomatitis virus as challenge and was performed on foreskin fibroblasts. This assay has been described previously (6, 7) and includes a standard assayed against NIH reference standard G-023-901-527.

Results. Tables I and II describe our results. No differences were appreciated between the quantity of interferon produced by fibroblasts in the presence of the antineoplastic agents or in the absence of these same drugs when I:C was used as the inducer. When fibroblasts were induced using NDV, only pretreatment with adriamycin was associated with any consistent reduction in interferon yield, but this reduction was of a minimal degree and its significance seemed slight. Methylprednisolone, 5FU, 6MP, and vincristine had no adverse effect on virus-induced interferon in fibroblasts even with pretreatment. All results were repeatedly reproduced in separate testing.

In contrast to the results with fibroblasts,

TABLE I. THE EFFECTS OF ANTINEOPLASTIC AGENTS ON INTERFERON PRODUCTION IN FIBROBLASTS

		Interferon titers								
		Antineoplastic agent present						Antineoplastic agent absent		
		Prior exposure			Simultaneous exposure					
Agent	Concentration	1	2	3	1	2	3	1	2	3
		I:C induction								
Vincristine	(0.08 μg/ml)									
Test 1		200	200	400	400	400	400	200	200	200
Test 2		3200	3200	3200	1600	3200	3200	3200	3200	3200
6-Mercaptopurine	(3.2 μg/ml)									
Test 1		200	200	200	200	400	400	400	400	400
Test 2		800	800	1600	800	800	800	800	800	1600
Adriamycin	(0.40 μg/ml)									
Test 1		800	1600	1600	400	800	800	1600	1600	1600
Test 2		200	200	200	200	200	200	200	200	200
5-Fluorouracil	(40.0 μg/ml)									
Test 1		800	800	1600	800	800	800	800	800	800
Test 2		1600	1600	1600	400	800	1600	400	800	800
Methylprednisolone	(80.0 μg/ml)									
Test 1		200	400	400	200	400	400	400	400	400
Test 2		400	800	800	400	400	800	400	400	800
		NDV induction								
Vincristine	(0.08 μg/ml)	'600	3200	—	3200	3200	—	3200	3200	—
6-Mercaptopurine	(3.2 μg/ml)	3200	3200	—	1600	3200	—	1600	3200	—
Adriamycin	(0.40 μg/ml)									
Test 1		1600	1600	—	800	3200	—	3200	3200	—
Test 2		1600	1600	—	3200	6400	—	6400	6400	—
Test 3		3200	—	—	6400	—	—	6400	—	—
Test 4		1600	—	—	3200	—	—	3200	—	—
5-Fluorouracil	(40.0 μg/ml)	800	1600	—	800	1600	—	1600	1600	—
Methylprednisolone	(80.0 μg/ml)	6400	6400	—	6400	6400	—	3200	6400	—

TABLE II. THE EFFECTS OF ANTINEOPLASTIC AGENTS ON INTERFERON PRODUCTION IN HUMAN MONONUCLEAR CELLS[a]

		Interferon titers					
		Antineoplastic agent present				Antineoplastic agent absent	
		Prior exposure		Simultaneous exposure			
Agent	Concentration	1	2	1	2	1	2
Vincristine	(0.08 μg/ml)						
Test 1		100	100	400	400	800	800
Test 2		400	800	800	1600	1600	1600
Test 3		200	200	—	—	800	800
6-Mercaptopurine	(3.2 μg/ml)						
Test 1		<100	<100	400	800	1600	1600
Test 2		<100	<100	400	800	800	800
Test 3		<100	<100	—	—	1600	1600
Adriamycin	(0.40 μg/ml)						
Test 1		1600	1600	800	1600	1600	1600
Test 2		800	800	400	800	800	800
5-Fluorouracil	(40.0 μg/ml)						
Test 1		800	1600	1600	1600	1600	1600
Test 2		800	1600	400	800	800	800
Methylprednisolone	(80.0 μg/ml)						
Test 1		400	800	400	800	800	800
Test 2		800	1600	800	1600	1600	1600

[a] NDV induction.

vincristine consistently lowered interferon yields when the leukocytes were pretreated with this drug, and 6MP, under the same conditions, virtually eliminated detectable interferon production. Both of the differences were significant ($P < 0.01$ by Student's t test). Simultaneous exposure for both drugs resulted in a diminished yield in three of the four tests. Adriamycin, methylprednisolone, and 5-fluorouracil failed to diminish mononuclear cell interferon yields. Again the results were reproducible in separate testing.

[Titration of NDV performed by hemagglutination before and after induction in both fibroblasts and mononuclear cells demonstrated that no change in virus concentration occurred during induction in either test or control flasks.]

Discussion. This report demonstrated that antineoplastic agents can influence interferon production by human cells *in vitro*. The observed alterations in interferon production, however, related not only to the particular drug in question but also to the cell type and the inducer.

We found the production of fibroblast interferon after I:C induction was unaltered by any of the antineoplastic agents studied. Furthermore, while adriamycin appeared to diminish the yield of fibroblast interferon if the cells were pretreated with the drug for 24 hr prior to induction with NDV, the difference in interferon yield was minimal. None of the other drugs studied affected virus-induced interferon production by fibroblasts. Thus, it seems fibroblasts were relatively resistant to the effects of the antineoplastic agents, at least as regards interferon production.

In contrast to the observations made with fibroblasts, vincristine and 6-MP lowered the quantity of interferon produced by mononuclear cells after viral induction. The reduced yields, however, were consistently seen only when mononuclear cells were pretreated with the antineoplastic agent. Prior treatment with vincristine reduced the quantity of interferon produced by as much as 88% and pretreatment with 6-MP virtually eliminated detectable interferon production.

In the circumstances where diminished interferon yields were demonstrated the question arises as to whether these effects occurred because of alterations in the host cell or because of effects on the virus. Virus titrations were performed by hemagglutination during induction and no viral proliferation could be documented under these circumstances. While infectivity measurements would have been a more reliable means of assessing viral proliferation, the fact that viral reproduction may not occur in this system suggests alterations in the cell are responsible for the observed diminution in interferon yields. Furthermore, if the effects were on the virus it would have been expected that the drugs would have *decreased interferon yields in both types of cells* used rather than just one as we described. It therefore seems likely that the diminished yields were due to alterations in the cell induced. It is probable that cell macromolecular synthetic capacities were compromised in those circumstances where diminished yields were documented. This hypothesis will be verified in future studies. Furthermore, it is likely that pretreatment permits the compromise in synthetic functions to occur before interferon production begins while simultaneous treatment permits the initiation of interferon synthesis before the drugs can affect the cell. Previous studies have provided a precedent for the assumption that protein-synthesizing capacities may be impaired at least by 6MP. Thus, Tidd and Paterson (8) have described alterations in RNA due to 6MP, and as both RNA (9, 10) and protein synthesis (11) are required for interferon production, chemicals which alter RNA could adversely influence interferon yield.

The fact that 6MP and vincristine altered production of interferon in mononuclear cells, but not fibroblasts, likely relates to the specific effects these drugs have on individual cell types. Thus, 6MP affects primarily leukemic cells (12) and vincristine affects differing cell types including those derived from lymphoidal origin (12).

The concentrations of chemotherapeutic agents used in these experiments were purposely selected to approximate the highest or peak therapeutic concentrations achieved in man. These high concentrations were used to maximize our opportunity to demonstrate any effect on interferon synthesis by these drugs. We have shown that at these peak therapeutic concentrations 6MP and especially vincristine can influence interferon production in mononuclear cells. Since, however, drug concentrations fall as the drug is eliminated by metabolism or excretion, it is now necessary to perform *in vivo* studies to find if the effect on interferon synthesis reported here can be demonstrated in the body. Further studies are currently in progress in our laboratory to find the minimum concentrations necessary to diminish interferon yields from mononuclear cells and to determine the duration of drug exposure necessary to affect this lessened production of interferon.

These studies offer some support to the hypothesis that the increased severity of viral infections occasionally observed during the course of neoplastic diseases could be at least in part due to diminished capacity of the patient to produce interferon in the presence of antineoplastic drugs. *In vivo* studies as mentioned above, however, will be necessary to confirm this hypothesis.

While no other comprehensive study similar to this investigation has been carried out on human cells, other relevant investigations deserve comment. Kilbourne *et al.* (13) using eggs, Postic *et al.* (14) using rabbits, and Talas and Stoger (15) using mice, all demonstrated diminished interferon production in the presence of steroids. In contrast, DeSomer *et al.* (16) working in rats found steroids failed to depress interferon levels and Mendelson *et al.* (17) found steroids actually increased interferon levels in mice.

As regards studies in tissue culture models, DeMaeyer and DeMaeyer (18) working on rat tumor cells found steroids depressed interferon production, but Adolf and Swetly (19) have recently found that various steroids can augment interferon production of human lymphoblastoid cells when induced by viruses.

Havell and Vilcek (20) have studied the effect of vinblastine, a drug related to vincristine, on interferon production and found

little adverse influence; however, these authors noted this drug diminished interferon secretion.

St. Geme *et al.* (21) investigated the effects of 6MP on the ability of chick embryo fibroblasts to produce interferon, and while documenting resultant yields were slightly diminished, these authors felt the differences were not significant.

The discrepancy between the results of some of these studies and our own are multifaceted, but likely result from inherent dissimilarities between the animals used and between the basic cell types studied.

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