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Inhibition of Interferon Yield by Vincristine[1,2] (41800)

GEOFFREY J. GORSE,*,[3] LEWIS M. SLATER,† HAROLD S. KAPLAN, J. G. TILLES, AND THOMAS C. CESARIO*

*Division of Infectious Disease and †Division of Hematology and Oncology of the Department of Medicine, University of California, Irvine Medical Center, Orange, California 92668

Abstract. Vincristine has been shown *in vitro* to adversely influence the interferon system. In order to further investigate the effects of vincristine on interferon production, we determined the dose of vincristine and the duration of time required to diminish Newcastle Disease Virus-induced human leukocyte interferon production *in vitro*. Further, mice were treated with vincristine and induced to produce interferon using 10-carboxymethyl-9-acridanone. The concentration required to diminish interferon yields was the very highest found in man during therapy. The duration of exposure to vincristine (at the highest concentration) required to influence interferon production far exceeded the duration this concentration persists in serum during therapy. Furthermore, the mouse model failed to show any diminished interferon producing capacity for vincristine treated mice. We conclude that while vincristine can clearly diminish interferon yields, current treatment regimens using vincristine probably do not alter interferon production. The mechanism by which vincristine influences interferon yields *in vitro* appears to be by a direct effect on the ability of the cell to produce interferon.

Immunosuppressive drugs are known to decrease host defense against viral infection (1, 2). Inhibition of interferon (IFN) production, as well as suppression of cellular and humoral immune mechanisms, could contribute to reduced host defense against viral infection (3). Knowledge of the effects of antineoplastic agents on IFN production is of importance as exogenous IFN could be supplied to patients with serious viral infections if it is found that such patients are deficient in interferon-producing capacities secondary to the effects of chemotherapy.

Several authors have reported diminished *in vitro* leukocyte IFN production by peripheral blood mononuclear leukocytes (PBML) obtained from patients undergoing immunosuppressive chemotherapy after organ transplantation. Rytel and Baley (4) reported mononuclear leukocytes obtained from renal transplant patients receiving treatment with steroids and azathioprine have yielded decreased quantities of IFN after *in vitro* induction with Newcastle Disease Virus (NDV) as compared to leukocytes obtained from healthy hospital personnel. Levin *et al.* (5) demonstrated that *in vitro* IFN production by PBML obtained from patients receiving bone marrow transplants and undergoing immunosuppression with steroids, methotrexate, and anti-thymocyte serum is diminished. These authors demonstrated this phenomenon in response to induction with NDV, cytomegalovirus antigen, and phytohemagglutinin (PHA) during the first 3 months post-transplantation. Similarly, Rand *et al.* (6) reported depression of *in vitro* PBML IFN production after induction with herpes simplex virus (HSV) or with varicella zoster virus (VZV) in cardiac transplant recipients during the first 3 months following transplantation. They found that IFN production in response to VZV antigen remains blunted greater than 6 months following transplantation. Herpes simplex and herpes zoster infections were more frequent in their transplant recipients compared to normal controls. In a recent study, Pollard *et al.* studied lymphocyte transformation and IFN production in response to HSV, CMV, and VZV antigens by PBML

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[3] To whom reprint requests should be addressed at: Section of Infectious Diseases, Department of Medicine, Marshall University School of Medicine, Huntington, W. Va. 25701.

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from cardiac transplant recipients receiving antithymocyte globulin, prednisone, and azathioprine (2). They found that IFN production in these patients in response to each of the three antigens is depressed for prolonged periods of time following transplantation. The results of these studies could be explained by *in vitro* effects of the various chemotherapeutic agents administered *in vivo* on PBML function. The presence or absence of effects caused by each individual agent on PBML IFN production is not clearly delineated by these studies.

In vitro studies performed by Cesario *et al.* demonstrate depressed leukocyte IFN production by PBML obtained from normal human donors, when the cells were treated with therapeutic concentrations of vincristine (VCR) or 6-mercaptopurine (6 MP) for 24 hr prior to and during induction with NDV (7). No effect on IFN yield is seen when human PBML are similarly treated with therapeutic concentrations of adriamycin, 5-fluorouracil, or methylprednisolone. In related experiments, overnight pretreatment of human fetal foreskin fibroblast (FF) monolayers with VCR, adriamycin or 6 MP, reduce the antiviral effect of both human alpha and beta IFN (8).

The objectives of the current study were to determine the concentration of VCR and the exposure time required to diminish IFN yields. In addition, the effects of VCR on *in vivo* mouse IFN production are reported.

Materials and Methods. The platelet-rich buffy coat fraction of human blood was obtained from normal donors at the American Red Cross. These preparations were 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 final concentration of 1×10^7 cells/ml in RPMI 1640 media containing 10% bovine fetal serum (BFS), 1 mg/ml dextrose, 30 μg/ml glutamine, 90 μg/ml arginine, 150 u/ml penicillin G, and 250 μg/ml streptomycin sulfate.

Vincristine (Eli Lilly, Indianapolis) was freshly prepared prior to use. The concentrations of VCR chosen for the human PBML experiments are at or below the serum concentration immediately following intravenous administration to humans (9).

NDV was grown in eggs using a laboratory strain that had been serially passed through many generations.

Induction of IFN in PBML was accomplished as follows. The cells were adjusted to a concentration of 1×10^7 cells/ml suspended in supplemented RPMI 1640 media. Test cells were exposed to media containing varying concentrations of VCR as noted for the appropriate period of time in a 37°C incubator. Control cells were simultaneously incubated in identical media not containing the VCR. All tests were performed in triplicate.

The cells were washed and resuspended in fresh media with or without the continued presence of VCR. NDV in a concentration of 100 EID_{50}/cell was added to each tube and incubation carried out overnight in a 37°C incubator. The cells were then centrifuged, and the supernatant harvested and dialyzed against 0.1 *M* citric acid (pH 2.7) for 4 days and PBS (pH 7.0) for 1 day. The dialysate was harvested, centrifuged for 90 min at 25,000 rpm, and frozen at −80°C for later assay. In order to release intracellular IFN, cells were resuspended in fresh media and subjected to five cycles of freeze–thawing, as modified from Havell and Vilcek (10). The lysed particulate cellular debris was centrifuged, and the supernatant collected and dialyzed as described above.

Viability counts were performed using a trypan blue dye-exclusion method and were greater than 90% in both test and control cells. The difference in viability between test and control cells was less than 10% in all cases.

Human IFN 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 (11, 12) and includes a standard assayed against NIH reference standard G-023-901-527. Assay of mouse IFN activity was also carried out in microtiter using mouse L cells and a challenge with vesicular stomatitis virus.

Egg infectivity titrations were done using embryonated chicken eggs inoculated in duplicate with serial 10-fold dilutions (10^{-5} through 10^{-10}) of the seed NDV, NDV incubated with PBML in the absence of VCR, and NDV incubated with PBML in the presence of VCR at a concentration of 0.08 μg/

ml. The eggs were incubated at 37°C for 48 hr, then refrigerated for 24 hr at 4°C. The allantoic fluid was harvested from each egg and NDV titer determined by hemagglutination with human red blood cells.

BDF/1 hybrid (Simonsen) mice, weighing 18–20 g, were treated in groups of five mice with VCR (30 μg/kg body wt). CMA (10-carboxymethyl-9-acridanone sodium salt) was injected subcutaneously. CMA is a known low-molecular-weight IFN inducer in mice (13). Two types of experiments were performed. In the first, mice were induced with CMA 2 and 24 hr after the dose of VCR. In a second set of experiments, mice were induced at 4 hr, and at 1, 5, 9, 13, and 21 days after VCR administration. Control mice were identically induced, but did not receive VCR.

Blood was obtained from mice 2 and 4 hours after CMA injection by retroorbital sinus puncture using capillary tubes containing ACD anticoagulant. Samples from each group of five mice were pooled, the serum extracted and stored at −80°C until assay for IFN activity was performed.

Results and Discussion. The minimum concentration of VCR necessary for inhibition of IFN production by PBML was determined. A consistent reduction in IFN yield occurred when the PBML were exposed to 0.08 μg/ml VCR for 24 hr prior to induction with NDV (Table I). No consistent reduction in IFN yield occurred when cells were exposed to lower concentrations of the drug (Table I); however, at 0.04 μg/ml an occasional effect on IFN production was seen in PBML from certain donors (Table II). At the 0.08-μg/ml concentration it was found that the presence of VCR was not required during the induction to diminish the IFN yield. Thus, if the VCR was removed from the cells after a 24-hr period of pretreatment, yield reduction still occurred.

The minimum duration of exposure to VCR for demonstration of the reduced IFN yield effect was also determined. PBML were treated with VCR at a concentration of 0.08 μg/ml for 1, 4, 12, or 24 hr (Table III). After the pretreatment, the cells were washed and resuspended in fresh media in the absence of VCR, and induced with NDV. Pretreatment with VCR for 12 and 24 hr resulted in a consistent reduction in IFN production, but exposure periods of 1 and 4 hr had either no effect (1-hr exposure) or only an occasional effect (4-hr exposure).

TABLE I. EFFECT OF VINCRISTINE (VCR) CONCENTRATION ON PBML INTERFERON PRODUCTION

Vincristine concn (μg/ml)	Tube no.	Interferon titer: Vincristine 24 hr pre-NDV	Interferon titer: Vincristine 24 hr preinduction and during induction
0.08	1	<100	
	2	<100	
	3	<100	
0.04	1	3200	1600
	2	1600	1600
	3	1600	3200
0.02	1	3200	3200
	2	1600	1600
	3	3200	1600
0.01	1	3200	1600
	2	3200	1600
	3	1600	1600
Control (No VCR)	1	800	
	2	1600	
	3	1600	

The mechanism of the VCR effect was addressed. VCR could influence IFN yields either by inhibiting synthesis of IFN, or by inhibiting secretion of intracellular IFN from the cell. Alternatively, the effect could be related to alterations in the virus inducer. Freeze–thaw experiments were therefore performed to determine if VCR treated cells contained intracellular IFN which could be released by cell disruption. No intracellular IFN was detectable within cells pretreated with VCR at 0.08 μg/ml for 24 hr or in simultaneous control cells treated only with NDV (Table II) and subsequently disrupted by freezing. Further, egg infectivity titrations of the NDV indicated that viral titers were unaffected by a 24-hr incubation with VCR. Furthermore, no viral proliferation occurred in either treated or control PBML during the phase of IFN induction. Thus, VCR appeared to exert its effect by some mechanism other than preventing the release of IFN and did not exert its effect by simply diminishing viral proliferation.

The concentration of VCR necessary for a

TABLE II. EFFECT OF VINCRISTINE (VCR) ON PBML INTERFERON PRODUCTION

VCR concn	Tube no.	Interferon titer: Vincristine 24 hr pre-NDV	Interferon titer: Vincristine 24 hr preinduction and during induction
Extracellular (μg/ml)			
0.08	1	80	80
	2	80	80
	3	40	80
0.04	1	160	160
	2	160	80
	3	320	80
0.02	1	640	
	2	320	
	3	320	
Control	1	640	640
	2	640	640
	3	640	640
Intracellular (μg/ml)			
0.08	1	0	0
	2	0	0
	3	0	0
Control	1	0	0
	2	0	0
	3	0	0

TABLE IV. EFFECT OF VINCRISTINE (VCR) ON PEAK INTERFERON TITERS IN MICE INDUCED WITH CMA[a]

Treatment	Serum interferon titer: Expt 1	Expt 2	Expt 3	Expt 4
CMA alone	≥1280	640	320	320
CMA 2 hr post-VCR	640	80	320	320
CMA 24 hr post-VCR	320	320	320	320
VCR alone	<10	<10	<10	<10
No treatment	<10	<10	<10	<10

[a] 10-carboxymethyl-9-acridanone.

consistent inhibition of IFN production was the highest serum level achieved clinically. Further, the minimum duration of treatment with VCR to reduce IFN titer was greater than 4 hr. Thus, while VCR reduced IFN yields *in vitro* based on the evidence presented, reduced IFN yields in man seem unlikely during chemotherapy with VCR, as the 0.08 μg/ml concentration is attained for a very brief period before the VCR levels plunge to much lower concentrations. In contrast to vinblastine, a related periwinkel alkaloid, VCR did not appear to influence IFN release (10, 14, 15). Pre-

TABLE III. EFFECT OF DURATION OF PRETREATMENT WITH VINCRISTINE (VCR) ON PBML INTERFERON PRODUCTION

Duration VCR exposure preinduction (0.08 μg/ml)	Tube no.	Interferon titer: Test 1	Interferon titer: Test 2	Mean percentage reduction from control: Test 1	Mean percentage reduction from control: Test 2
1 hr	1	3200	800	0	0
	2	3200	800		
	3	3200	1600		
4 hr	1	1600	800	58	0
	2	1600	800		
	3	800	800		
12 hr	1	20	50	99	93
	2	20	100		
	3	40	10		
24 hr	1	200	50	92	95
	2	400	50		
	3	200	20		
Control (No VCR)	1	3200	800		
	2	3200	800		
	3	3200	800		

TABLE V. EFFECT OF VINCRISTINE (VCR) ON PEAK INTERFERON TITERS IN MICE INDUCED WITH CMA[a] AT LONG INTERVALS AFTER VCR DOSAGE

Treatment	Serum interferon titer
CMA alone	320
CMA 4 hr post-VCR	640
CMA 1 day post-VCR	320
CMA 5 days post-VCR	320
CMA 9 days post-VCR	160
CMA 13 days post-VCR	320
CMA 21 days post-VCR	160
No treatment	<10

[a] 10-carboxymethyl-6-acridanone.

sumably, VCR influenced IFN production. This is consistent with the known effect of VCR on protein synthesis (15).

The *in vivo* effects of VCR on mouse IFN production were investigated. While early experiments indicated that VCR might reduce IFN production in this animal model, subsequent experiments failed to demonstrate consistent reduction in IFN titer in VCR treated mice (Tables IV and V). The mechanism by which CMA induces IFN is unknown and it may be different from that of true viruses. VCR could possibly reduce IFN yields during viral infection, but not during administration of CMA. In the murine model of IFN induction using this drug, however, the available evidence is compatible with the *in vitro* data and suggests that therapeutic concentrations of VCR will not diminish IFN yields *in vivo.*

Thus, while VCR provides a useful tool to manipulate IFN production *in vitro,* it is not at all clear that it has a significant effect on IFN yields *in vivo.*

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