INHIBITION OF INTERFERON SECRETION BY VINBLASTINE

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The plant alkaloids vinblastine and colchicine are known to arrest cells in mitosis by virtue of their binding to spindle protein. These drugs are also capable of binding to microtubule protein and causing these structures to disaggregate into non-functional subunits (1, 2). Microtubular structures are thought to be involved in the secretory process of a number of proteins including insulin (7), collagen (4), and thyroid hormone (12). In this report we present our findings on the effects of these two drugs on the synthesis and secretion of interferon in a high producing human foreskin fibroblast strain (FS-4) (11).

MATERIALS AND METHODS

A human diploid cell strain (FS-4), derived in this laboratory from a single neonate foreskin, was used throughout. All experiments were carried out in cultures grown in 60-mm plastic petri dishes (Falcon Plastics, Los Angeles, Calif.). Each dish was seeded with 200,000 cells in 5 ml Eagle's minimal essential medium (MEM) buffered with HEPES (6 mM), Tricine (13 mM), and sodium bicarbonate (1.1 g/liter), supplemented with 5% heated fetal bovine serum. The medium was changed for fresh growth medium on the 6th day and the cultures were used for experiments at 11–13 days after seeding. The incubation was carried out in a humidified CO_2 incubator at 37°C.

The microassay for human interferon was described in detail earlier (6). In short, wells of the Micro Test II plastic tissue culture plates (Falcon Plastics, or Linbro Corp., New Haven, Conn.) were filled with 100 μl of MEM containing 5% fetal calf serum, and duplicate serial twofold dilutions of the assayed materials were prepared with the aid of an automatic micropipette. To each well was then added a suspension of human foreskin cells (30,000 cells/well in 100 μl of MEM as above). Indiana-type vesicular stomatitis virus (1,000 PFU/well) was added to each well after 18–20 h incubation, and a final reading of the test was taken by microscopic examination about 48 h after virus inoculation. The reciprocal of the highest dilution of the assayed sample which protected at least 50% of the cell sheet from the cytopathic action of the virus was taken as the interferon titer. All interferon titers throughout the paper are expressed in terms of reference units per milliliter, based on the 69/19 reference standard for human interferon (obtained from Antiviral Substances Program, Infectious Disease Branch, National Institute of Allergy and Infectious Diseases, Bethesda, Md.).

Before assaying for interferon, samples obtained from cultures treated with vinblastine or colchicine were dialyzed to remove the alkaloids. Dialysis was done at 4°C against two changes of phosphate-buffered saline,

pH 7.4, followed by final dialysis against MEM. It was established that the incubation of an interferon preparation with vinblastine (10^{-4} M) or colchicine (10^{-3} M) for 1 h at 37°C, followed by dialysis in order to remove the alkaloids, did not alter the antiviral activity of interferon.

Polyinosinate-polycytidylate (poly I·poly C) was supplied by the Antiviral Substances Program. Colchicine was obtained from the Sigma Chemical Co., St. Louis, Mo. Vinblastine sulfate (Velban) was purchased from Eli Lilly and Company, Indianapolis, Ind. Stock solutions of colchicine and vinblastine were prepared in MEM and kept frozen at −20°C until used.

RESULTS

Exposure of FS-4 cultures to the inducer poly I·poly C leads to rapid intracellular interferon synthesis, with the maximum rate of accumulation in the extracellular fluids reached at about 3 h and a rapid diminution of synthesis thereafter (see control group in Fig. 1). At concentrations of 10^{-4} M, both colchicine and vinblastine inhibited the appearance of interferon in the culture fluids (Table I). When the inhibitors were removed at 6 h and the cultures were reincubated with drug-free medium, reversal of the inhibitory action occurred in vinblastine-treated cultures, but not in colchicine-treated cells.

TABLE I

Effects of Colchicine and Vinblastine on Interferon Production

Treatment*	Interferon yield‡	
	6 h	24 h
	U/ml	
None	32	4
Colchicine		
10^{-3} M	<4	<4
10^{-4} M	6	2
10^{-5} M	24	4
Vinblastine		
10^{-4} M	6	96
10^{-5} M	32	6

* FS-4 cells were induced with 100 μg/ml of poly I·poly C in 1 ml of MEM for 30 min. Thereafter the cells were thoroughly washed and replenished with 2 ml serum-free MEM containing the designated concentrations of either of the two drugs.

‡ At 6 h after the start of induction the fluids were collected, and the cultures were washed and reincubated for an additional 18 h with 2 ml of MEM containing 2% fetal bovine serum without alkaloids. All culture fluids were exhaustively dialyzed to remove the alkaloids and then titrated for their interferon content.

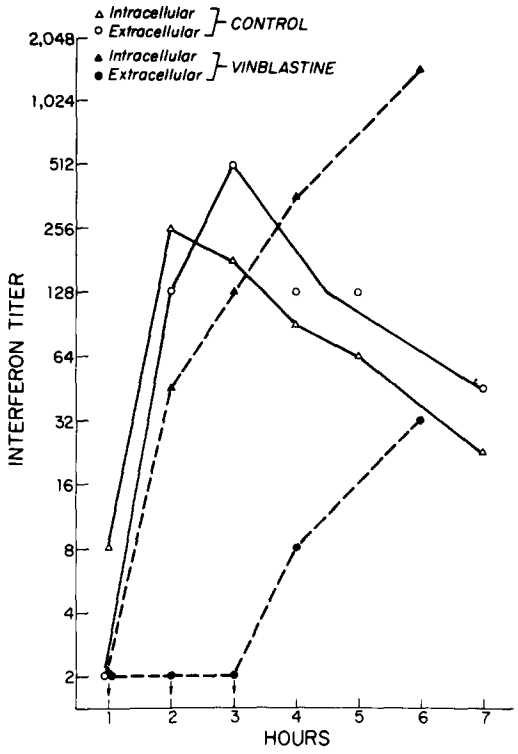

FIGURE 1 Effect of vinblastine (10^{-4} M) on the intracellular appearance of interferon and its secretion. Cultures were induced as in Table I, except that MEM with 2% fetal bovine serum was added at 30 min. For the determination of intracellular interferon levels, cultures were processed at intervals as described in Table II, except that freeze-thawing was done in 2 ml MEM with 2% fetal bovine serum. For the determination of extracellular interferon levels, medium from the same two treated and control cultures each was collected at intervals; after collecting the medium the cells were quickly washed once with 37°C warm medium, replenished with 2 ml of fresh 37°C warm maintenance medium (with or without vinblastine), and immediately returned to the incubator until the next interval. Vinblastine was present in the experimental group throughout the duration of the experiment. Interferon titers in the extracellular fluids collected at 6 or 7 h were divided by two to correct for the longer interval that had elapsed before the last harvesting period. Thus all extracellular interferon titers actually represent cumulative yields per hour, whereas intracellular interferon titers represent the net amount of interferon recovered at the particular interval from the cell extracts, after dilution in the 2 ml of medium added before freeze-thawing. Arrows indicate that interferon titers were less than 2. All samples were exhaustively dialyzed before the interferon assay.

Colchicine has a wide variety of effects on cells, including the inhibition of DNA synthesis (5). Interference with the uptake of nucleosides in WI-38 cells was also reported (8). In order to determine whether the inhibitory action of colchicine was due to the suppression of some early event in interferon induction, rather than of its secretion, the drug was added either together with the inducer or at different times after the removal of poly I·poly C from the cultures. Only when added along with the inducer or immediately after its removal at 0.5 h did colchicine cause inhibition of interferon production, demonstrated by reduced accumulation both in the culture fluids and intracellularly (Table II). Since extracellular interferon remains undetectable until about 2 h after induction (Fig. 1), this finding indicates that the action of colchicine is not on secretion of interferon, but rather, on an earlier event, most likely the processing of inducer or the synthesis of interferon messenger RNA.

TABLE II

Effect of Colchicine Added at Various Times Before and After Interferon Induction with Poly I·Poly C

h of colchicine (10^{-3} M) addition to cells*	Interferon yield‡		
	6 h		24 h
	Extracellular	Intracellular	Extracellular
		U/ml	
Control (no drug)	512	24	24
0	16	<8	<8
0.5	32	<8	<8
1.5	512	24	24

* At 0 h all cultures received 100 μg/ml of poly I·poly C with or without colchicine. 30 min later the inducing agent was removed, the cultures were washed and then reincubated with 2 ml of MEM containing 2% fetal bovine serum. (The presence of serum during this period of incubation accounts for the difference in control interferon yields between this experiment and the experiment shown in Table I.) Colchicine was added at the designated times to the fluids and kept on the cultures until 6 h.

‡ At 6 h the fluids from each group were collected and one-half of the plates from each group was washed and reincubated with MEM containing 2% fetal bovine serum until 24 h. The remaining plates of each group were washed with ice-cold saline and 1 ml of ice-cold MEM containing 2% fetal bovine serum was added to each plate. The cultures were then subjected to 5 cycles of rapid freezing in a dry ice-alcohol bath and thawing at 37°C. The resulting homogenates were dialyzed and assayed for intracellular interferon. All other procedures were as in Table I.

Vinblastine, on the other hand, selectively inhibited the secretion of interferon while not preventing its intracellular synthesis (Fig. 1). Both the intracellular and extracellular interferon yields were determined at various intervals after induction in vinblastine-treated and control cells. In the control cells intracellular levels were maximal at 2 h and release into the culture fluid peaked between 2 and 3 h after induction. In vinblastine-treated cultures the appearance of intracellular interferon was slightly delayed initially. However, at later intervals the release of interferon was greatly delayed and diminished, while intracellular levels continued to rise until at least 6 h. It is of interest that the total amount of interferon produced in vinblastine-treated cells was about two- to threefold greater than in control cultures. This effect may be related to the known "superinducing" action of inhibitors of RNA or protein synthesis on interferon production in this system (11).

DISCUSSION

Interferon secretion from rabbit kidney cells was shown to be both an energy- and temperature-dependent process (9). *p*-hydroxy-mercuribenzoate inhibited the release of interferon, presumably by modifying SH groups on protein(s) or enzyme(s) thought to be required for the secretion of interferon (10). Because of the inhibitory effect of vinblastine on interferon secretion, it would be tempting to conclude that microtubules are in some way involved in the release of interferon from the cell, since it has been suggested that many secreted proteins, from a variety of cells, rely on the microtubular network for their export. For example, collagen release in chick frontal bone and 3T3 fibroblasts was inhibited by both colchicine 10^{-5} M and vinblastine 10^{-5} M and the authors attributed the impairment of secretion to disruption of microtubules responsible for the transport of collagen out of the cell (4).

In contrast, only high concentrations of vinblastine prevented the secretion of interferon from the FS-4 fibroblasts, and colchicine, even in high concentrations, was ineffective in inhibiting the release process. The binding sites on the microtubule protein components for these two drugs

were shown to be different, with vinblastine binding to a site which can also bind calcium (13). Dales and Chardonnet (3) showed that vinblastine is effective in disrupting microtubules which transport adenovirus from the cytoplasm to the nuclear membrane for uncoating, whereas colchicine had no effect on virus transport. Although the involvement of microtubular structures in the inhibitory action of vinblastine on interferon secretion remains uncertain, the selective disruption of the secretion process, without inhibition of synthesis, could prove useful in studies of the intracellular synthesis and processing of interferon.

SUMMARY

At concentrations of 10^{-4} M both colchicine and vinblastine inhibited interferon production in the FS-4 strain of human foreskin fibroblasts stimulated with polyinosinate-polycytidylate (poly I·polyC). Colchicine interfered with an early event, most likely the processing of inducer or the synthesis of interferon messenger RNA, while not affecting the secretion of interferon. Vinblastine, on the other hand, caused a preferential inhibition of interferon secretion.

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REFERENCES

1. ADELMAN, M. R., G. G. BORISY, M. L. SHELANSKI, R. C. WEISENBERG, and E. W. TAYLOR. 1968. Cytoplasmic filaments and tubules. *Fed. Proc.* **27**:1186–1193.
2. BENSCH, K., and S. MALAWISTA. 1968. Microtubule crystals: a new biophysical phenomenon induced by vinca alkaloids. *Nature (Lond.).* **218**:1176–1177.
3. DALES, S., and Y. CHARDONNET. 1973. Early events in the interaction of adenovirus with HeLa cells. IV. Association with microtubules and nuclear pore complex during vectorial movement of the inoculum. *Virology.* **56**:465–483.
4. DIGELMANN, R. F., and B. PETROKOFSKY. 1972. Inhibition of collagen secretion from bone and cultured fibroblasts by microtubular disruptive drugs. *Proc. Natl. Acad. Sci. U. S. A.* **69**:892–896.
5. FITZGERALD, P. H., and L. A. BREHAUT. 1970. Depression of DNA synthesis and mitotic index by colchicine in cultured human lymphocytes. *Exp. Cell Res.* **59**:27–31.
6. HAVELL, E. A., and J. VILČEK. 1972. Production of high titered interferon in cultures of human diploid cells. *Antimicrob. Agents Chemother.* **2**:476–484.
7. LACY, P. E., S. L. HOWELL, D. A. YOUNG, and C. J. FINK. 1968. New hypothesis of insulin secretion. *Nature (Lond.).* **219**:1177–1179.
8. MIZEL, S. B., and L. WILSON. 1972. Nucleoside transport in mammalian cells. Inhibition by colchicine. *Biochemistry.* **11**:2573–2578.
9. TAN, Y. H., J. A. ARMSTRONG, and M. HO. 1971. Intracellular interferon: kinetics of formation and release. *Virology.* **45**:837–840.
10. TAN, Y. H., D. K. JENG, and M. HO. 1972. The release of interferon: an active process inhibited by *p*-hydroxymecuribenzoate. *Virology.* **48**:41–48.
11. VILČEK, J., and E. A. HAVELL. 1973. Stabilization of interferon messenger RNA activity by treatment of cells with metabolic inhibitors and lowering of the incubation temperature. *Proc. Natl. Acad. Sci. U. S. A.* **70**:3909–3913.
12. WILLIAMS, J. A., and J. WOLF. 1970. Possible role of microtubules in thyroid secretion. *Proc. Natl. Acad. Sci. U. S. A.* **67**:1901–1908.
13. WILSON, L., J. BRYAN, A. RUBY, and D. MAZIA. 1970. Precipitation of proteins by vinblastine and calcium ions. *Proc. Natl. Acad. Sci. U. S. A.* **66**:807–814.