Interferon Induction in Normal and Leukemic Human Lymphocyte Cultures by Tilorone Hydrochloride[1] (36870)

ANTHONY J. DENNIS, HENRY E. WILSON, ANNA D. BARKER, AND MELVIN S. RHEINS
(Introduced by R. St. Pierre)

Department of Microbiology (College of Biological Sciences), Division of Hematology and Oncology (College of Medicine), The Ohio State University, Columbus, Ohio 43210

While several viral and nonviral agents have been shown to induce interferon in cultures of normal human leukocytes (1–3), leukocyte cultures from patients with chronic lymphocytic leukemia do not produce comparable levels of interferon when challenged with a viral inducer (4). Thus, it seemed appropriate to examine the interferon inducing capacity of a nonviral inducer (tilorone hydrochloride) in lymphocyte cultures from these patients.

Despite reports that tilorone hydrochloride (2–7 *bis* (diethylamino-ethoxy) fluoren-9-one dihydrochloride) has been shown to be an effective interferon inducer in murine leukemia models (5), it has not been tested in cultures of human lymphocytes. This class of low molecular-weight interferon inducers is of interest clinically due to their antiviral activity (6), and interferon induction in animals following oral administration (7).

Materals and Methods. Lymphocyte cultures. Leukocytes from three normal donors and from three patients with chronic lymphocytic leukemia (CLL) were obtained by leukapheresis (A. I. C. "Celltrifuge"). The diagnoses of CLL were established by accepted criteria (Dr. H. E. Wilson).

Ten ml quantities of the cell suspensions (37°) were transferred to sterile plastic screw-capped tubes, and 1.0 ml of Plasmagel (Laboratoire Roger Bellon) was added to each tube. Following a 15 min sedimentation period (room temperature), the buffy coat was removed and contaminating erythrocytes were eliminated by hypotonic lysis with the addition of cold 0.35% saline. After centrifugation, the cells were re-suspended in Eagle's Minimum Essential Medium with Spinners salts (MEM-S), supplemented with 10% heat-inactivated fetal-calf serum and 100 units of penicillin per ml. All cell counts were performed in a hemocytometer, with trypan blue exclusion employed as the test of viability. The cell concentration was adjusted to 1.0×10^9 viable cells/ml, and the lymphocytes were extracted by incubating the suspensions on pre-warmed (37°) 0.2 mm glass bead columns for 20 min. The lymphocytes were eluted with a volume of MEM-S equal to the internal void volume of the column. The resulting suspensions (approximately 98% lymphocytes) were dispensed in 100 ml aliquots (1.0×10^7 cells/ml) into 250 ml screw-capped flasks, and preincubated for two hr at 37° on an incubator-shaker prior to treatment with tilorone hydrochloride.

Inducer. Tilorone hydrochloride[2] was suspended in MEM-S without calf serum and sterilized by passage through a 0.22 μ Millipore filter. The inducer was added at concentrations of 0.01 μg/ml, 0.1 μg/ml, 0.5 μg/ml, 5.0 μg/ml and 50.0 μg/ml per 100 ml of lymphocyte suspension. Each treatment sample was prepared in triplicate, and 5.0 ml samples were removed at 8, 24 and 48 hr, stored at −70°, and subsequently assayed for interferon. Additionally, tests for cell viability and morphologic changes in the cell cultures were conducted at each of the test periods.

Interferon assay. Supernates of centrifuged samples from treated and from untreated control lymphocyte cultures were acidified to pH 2.0, held for 24 hr at 4°, and subsequently neutralized for the interferon assays.

Interferon was assayed by the plaque technique described by Merigan (8). Primary

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human foreskin fibroblasts served as the indicator cell monolayers, and the Indiana strain of bovine vesicular stomatitis virus as the challenge. Plaques were read at 48 hr, and the titers were recorded as the reciprocals of the dilutions of the cell-culture supernates which effected a 50% reduction in the number of plaques.

Results. In Figs. 1, 2 and 3 are represented the results obtained at the 8, 24 and 48 hr sampling periods. At each test period, the influence of inducer concentration on cell viability and morphology, and on interferon release was measured for both the normal and the leukemic lymphocyte preparations. Direct application of tilorone hydrochloride to foreskin cultures elicted no detectable interferon response.

By the 8 hr sampling period (Fig. 1), the viability of the normal lymphocytes had declined rapidly over the range of inducer concentrations between 0.5 μg/ml through 5.0 μg/ml, with a concomitant increase in the interferon titers (*i.e.*, 8 to 64) noted in the supernates. In contrast, leukemic cells exhibited neither a significant decline in viability nor an increase in interferon titers at these same inducer concentrations.

Progressive degenerative changes were exhibited in both the normal and the leukemic lymphocytes, but these were most evident in

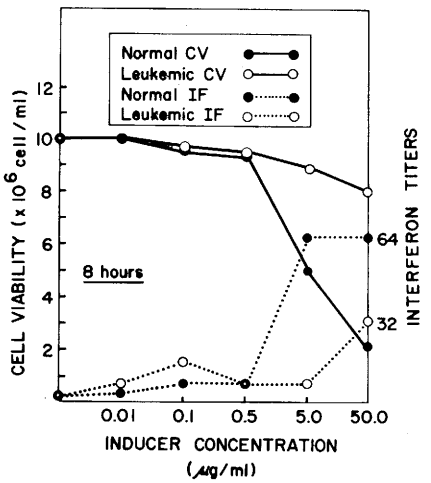

FIG. 1. The effect of varying concentrations of tilorone hydrochloride on cell viability and interferon release in normal and leukemic lymphocyte cultures at 8 hr.

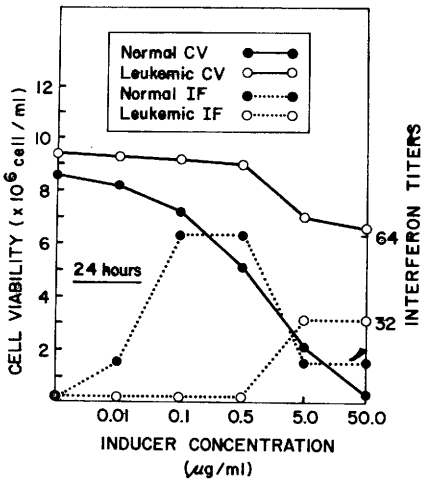

FIG. 2. The effect of varying concentrations of tilorone hydrochloride on cell viability and interferon release in normal and leukemic lymphocyte cultures at 24 hr.

the normal cultures, at all inducer concentrations. Most often seen was an increased number of cells with pycnotic nuclei, an acid shift in cytoplasmic staining, and an increased vacuolization (Wrights–Giemsa stain). An increase in lymphocyte fragility also was evidenced by the presence of large numbers of smudge cells.

The most prominent change noted in the leukemic cells was a cytoplasmic acid shift, which occurred in the cells treated with the higher inducer concentrations (*i.e.*, 5.0 μg/ml and 50.0 μg/ml).

By 24 hr, cell viability had declined from 8.6×10^6 cells/ml in the untreated control cultures to 2.0×10^4 cells/ml in those normal cell cultures which had been treated with the highest concentration of the inducer tested (*i.e.*, 50.0 μg/ml) (Fig. 2). By this period, a peak interferon titer of 64 was noted in the supernates from the normal cultures treated with 0.1 μg/ml of the inducer, as compared with the same titer obtained at 8 hr, with the use of 50 times this concentration. This increase was accompanied by an initial decrease, and a subsequent steady decline, in lymphocyte viability. Cellular degeneration and increased fragility were more apparent at this 24 hr period than was observed at the earlier test period.

A decline in viability also was noted in the

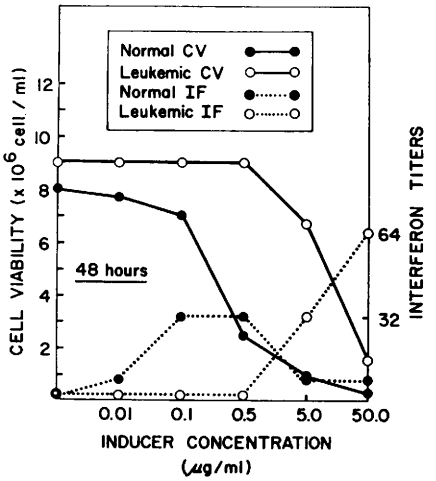

FIG. 3. The effect of varying concentrations of tilorone hydrochloride on cell viability and interferon release in normal and leukemic lymphocyte cultures at 48 hr.

leukemic cell cultures at 24 hr, and was first seen in the cultures treated with the 0.5 μg/ml drug concentration. The viability of the untreated leukemic lymphocyte suspensions was 9.4×10^6 cells/ml, while the viability of those cultures which had been treated with 5.0 μg/ml of inducer was reduced to 7.0×10^6 cells/ml. This decrease in viability was coincident with a five-fold increase in the interferon titer.

The peak interferon titer was 32 (Fig. 3) in the normal cell cultures from the 48 hr test samplings. Cell death was more extensive in all of the normal cell cultures, and approached 100% in cultures which had been treated with 50.0 μg/ml of the inducer.

At this same period, the cell viability in the leukemic cell cultures remained stable (9.0×10^6 cells/ml) over the range of inducer concentrations to 0.5 μg/ml. Supernates from these cultures contained no detectable interferon, while leukemic cell cultures which had been treated with 50.0 μg/ml of tilorone hydrochloride demonstrated a titer of 64.

Discussion. A direct relationship between increased interferon release and/or production and decreased cell viability was observed at all of the test periods for both the normal and leukemic lymphocyte cultures. This suggests that the interferon response elicited by tilorone hydrochloride treatment of the cultures was directly related to the degree of drug toxicity.

The morphological changes, decreased cell viability, and initiation of an interferon response were delayed in the leukemic cell cultures, indicating a possible depression of cellular activity, as compared with normal lymphocytes. Indeed, lymphocytes of these three leukemic donors were shown to have significantly lower rates of glycolysis and hexose-monophosphate shunt activity as compared with normal cells (9). This is also consistent with the general observation that chronic leukemic lymphocytes exhibit a delayed response to the mitogenic action of phytohemagglutinin (10), as well as reduced ability to produce interferon when exposed to Sendai virus (4). Although delayed, the regimen of treatment of leukemic cell cultures with the tilorone hydrochloride concentrations reported here, did result in the release of interferon in quantities comparable to those recorded for normal lymphocyte cultures.

The acid shift in cytoplasmic staining and the increased vacuolization suggest some limited intercytoplasmic action of the drug. Kaufman *et al.* reported an observation regarding the apparent storage and crystallization of tilorone hydrochloride in human corneal cells, following topical application of a 20% solution (11).

Summary. Lymphocytes recovered from normal donors and from patients with CLL were examined for interferon-producing capacity, following treatment with tilorone hydrochloride *in vitro*. The interferon response observed in normal lymphocyte cultures appeared to be correlated with the toxic effect of the inducer. The effect observed in the leukemic lymphocyte cultures was delayed, but of a similar magnitude. The data indicate that leukemic cells are either more resistant to the toxic effects of this agent, or their metabolic activity is less than observed in normal lymphocytes.

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