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Cytotoxicity and Cell Cycle Specificity of Homoharringtonine

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The plant alkaloid, homoharringtonine, isolated from *Cephalotaxus harringtonia* is cytotoxic to HeLa, KB, and L cells growing in monolayer cell cultures. This effect appears to be cell-cycle specific. In synchronized KB cells, protein synthesis was preferentially inhibited in the G_1 and G_2 phases (70 and 45% inhibition, respectively) as might be expected for a protein-synthesis inhibitor.

Homoharringtonine is an alkaloid that was first isolated from *Cephalotaxus harringtonia* (12). Its structure and the structure of related alkaloids have been established by Powell et al. (15). Several of these alkaloids have been found to be active against leukemia cell lines L1210 and P388 (10, 12-15). Since these compounds are of potential clinical use, it appeared interesting to establish the mechanism of homoharringtonine action. More recently, the mode of action of harringtonine was investigated (9). This compound inhibited both protein synthesis in HeLa cells and initiation of protein synthesis in rabbit reticulocyte homogenates. The present study was undertaken to examine the cell-cycle specificity of homoharringtonine in synchronized KB cells.

Cell lines were purchased from the American Type Culture Collection. Human oral epidermoid carcinoma cells (KB cells) were grown in monolayer cell cultures with basal Eagle medium supplemented with 10% heat denatured (57°C) fetal calf serum and containing 100 U of penicillin and 100 μg of streptomycin per ml (6). Human cervical carcinoma cells (HeLa cells) and murine fibroblast cells (L cells) were grown in a monolayer using Eagle minimum essential medium supplemented with 10% calf serum (heat denatured, 57°C) and containing 100 U of penicillin and 100 μg of streptomycin per ml (7, 8). Cells were maintained in the log phase of growth, at 37°C in an atmosphere of 5% CO_2 in air, and subcultured every 2 or 3 days. For experiments, cells were detached from the glass support with trypsin–ethylenediaminetetraacetic acid, collected by centrifugation and suspended in fresh medium, plated at a density of 1×10^5 to 5×10^5 cells/ml, and incubated for 3 h at 37°C to allow attachment to the support before an experiment was initiated.

Addition of various concentrations of homoharringtonine to cultures of KB, HeLa, and L cells showed that KB cells are most sensitive to homoharringtonine, as measured by cell growth (11) (Fig. 1). At a concentration of 6.7 ng/ml, growth was inhibited after 48 h, and cell death occurred after 72 h. At higher concentrations (13.3 ng/ml), cell death occurred immediately, and apparent complete cell death was observed after 24 h.

Both HeLa and L cells were less sensitive to homoharringtonine than were KB cells. The lowest concentrations of homoharringtonine required to cause cell death of the HeLa or L cells was 0.5 μg or 0.25 μg/ml, respectively. At these concentrations, the effect on HeLa cells appeared immediately; however, L cells required a 48-h exposure until cell death occurred.

Synthesis of macromolecules was monitored by measuring the incorporation of radioactively labeled thymidine, uridine, and lysine into acid-insoluble fractions. As reported previously (9), homoharringtonine preferentially inhibited protein synthesis in all three cell lines.

The cell-cycle specificity of homoharringtonine was investigated in KB cells. Cell populations synchronized in the S phase were obtained by a double-thymidine blockade (3, 4, 16-18). KB cells released from a double-thymidine arrest immediately entered S phase (Fig. 2). Maximum deoxyribonucleic acid (DNA) synthesis occurred after 4 h. A second pulse of DNA synthesis occurred after 22 h. Most mitotic figures (13%) were present after 12 to 13 h. From these results, KB cells were judged to be in the S phase after 1 h upon removal of the thymidine block and remained in the DNA synthetic phase for 3 to 4 h. The G_2 phase lasted for 3 to 4 h. Cells were in the M phase for 4 h (10 to 13 h after removal of the thymidine block) and in the G_1 phase for 5 to 6 h (13 to 19 h after removal of the thymidine block). This traverse through the cell cycle agreed well with published data (4).

KB cells in the different phases were then treated with homoharringtonine, and protein

synthesis was measured. Only limited cell-cycle specificity of homoharringtonine was found (Fig. 2 and 3). However, preferential inhibition of [^{3}H]lysine incorporation into acid-insoluble material was found to occur in the G_2 phase and especially in the G_1 phase. Since homoharringtonine is a protein-synthesis inhibitor and the greatest amount of protein synthesis occurs in the G_1 phase, and to a lesser extent in the G_2 phase, of the cell cycle, the results in Fig. 2 are as predicted, and are in good agreement with the cycle specificity of puromycin and cycloheximide (2). Both drugs are protein-synthesis inhibitors and exhibit a G_1 and a G_2 block.

Survival of synchronized cells was measured by suspending washed cells in fresh medium after the cells had been treated with 60 μg (11×10^{-8} M) of homoharringtonine per ml for 1 h. The cell suspension was diluted 1:1000, plated into dishes, and incubated at 37°C for 3 to 7 days. The number of colonies was determined under a microscope after staining with Giemsa. Most cell deaths (50%) occurred when KB cells in the G_1 phase were treated with homoharringtonine (Fig. 3). However, 67 and 75% of the cells in the G_2 and M phases, respectively, and nearly 100% of the cells in S phase survived the cytotoxic action of homoharringtonine. These results indicate that the effect of homoharringtonine on intact G_1-phase cells is longer lasting than the inhibition of cell-free protein synthesis of harringtonine (9). This process appeared to be readily reversible.

In general, these data indicate that homoharringtonine could be effective in combination chemotherapy against slow-growing tumors that have a larger proportion of their cells in the G_1 (or G_0) phase (1, 5). However, protein-synthesis inhibitors tend to show a high degree

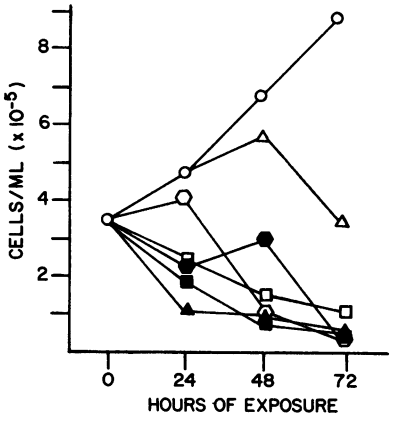

FIG. 1. *Effect of continuous exposure to various levels of homoharringtonine on cells growing in culture.* (○) *Control;* (△) *KB cells exposed to 6.7 ng/ml;* (▲) *KB cells exposed to 13.3 ng/ml;* (□) *HeLa cells exposed to 0.5 μg/ml;* (■) *HeLa cells exposed to 1.0 μg/ml;* (◇) *L cells exposed to 0.25 μg/ml;* (◆) *L cells exposed to 1.0 μg/ml. Each point represents the average of five measurements.*

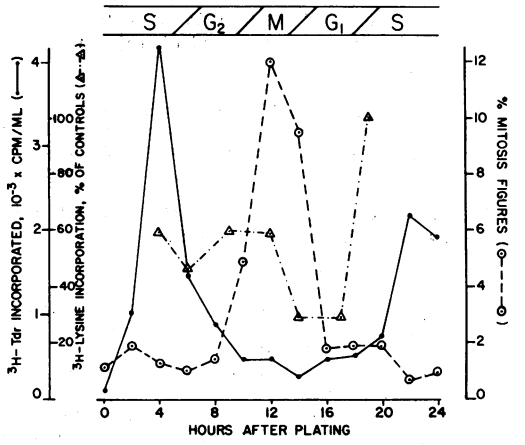

FIG. 2. *Traverse of KB cells through the cell cycle after a double-thymidine block. Thymidine block was removed at zero hours after plating. Cell-cycle phases were identified through the incorporation of [^{3}H]thymidine* (•) *into DNA and through counting of the mitotic figures* (⊙) *after staining with hematoxylin. Effect of homoharringtonine (30 μg/ml for 30 min) on the metabolic viability of KB cells in different phases of the cycle was measured by the incorporation of [^{3}H]lysine* (△) *into protein reported as percentage of control incubations. Typical values for control incorporation of [^{3}H]lysine into protein were: S phase, 1,300 cpm/mg of protein; G_2 phase, 1,820 cpm/mg of protein; M phase, 850 cpm/mg of protein; G_1 phase, 2,210 cpm/mg of protein. Each point on the graph represents the average of three measurements.*

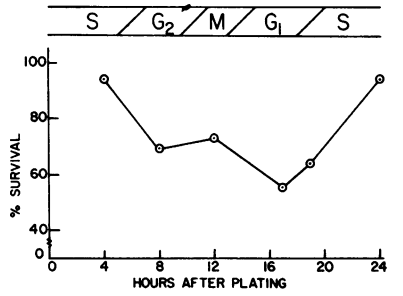

FIG. 3. *Effect of homoharringtonine on the survival of synchronized KB cells. Synchronized S-phase KB cells were incubated for 1 h with 60 ng of homoharringtonine per ml at 4, 8, 12, 17, 19, and 24 h after removal of the thymidine block, diluted 1:1000, and grown in monolayers for 7 days. Thereafter stained colonies were counted and compared with control incubations without homoharringtonine. Each point represents the average of three measurements.*

of toxicity that could severely limit or prevent their use in the management of human malignant disease.

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