Lethal Effect of Adriamycin on the Division Cycle of HeLa Cells

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SUMMARY

Adriamycin, a new antitumor antibiotic in the anthracycline group, promptly inhibits DNA and RNA synthesis and arrests cell division. The cell viability (defined as the capacity of a single cell to grow out into a macroscopic clone) is reduced sharply following exposure to adriamycin, 0.1 μg/ml, for a fractional period of the generation time. With the use of a synchronous population of HeLa cells, it is shown that the maximum loss in cell viability takes place when exposure to adriamycin occurs during the DNA-synthetic phase (S). The relative dose-response curves of HeLa cells exposed to either adriamycin or daunomycin show that daunomycin is significantly more effective in reducing the cell viability than is adriamycin on a molar basis.

INTRODUCTION

Adriamycin is an antibiotic of the anthracycline group isolated from Streptomyces var. caesius (1). The antibiotic has a chemical structure similar to that of daunomycin, differing from daunomycin only in the replacement of a hydrogen atom in the acetyl radical of the aglycone moiety by a hydroxyl group (9). Adriamycin has recently been reported to be an effective growth inhibitor of several human tumors as well as of leukemic cells. Preliminary clinical studies seem to indicate that the adriamycin might have a higher therapeutic index (the ratio of the normal tissue tolerance dose to the tumor lethal dose) than does daunomycin (2, 7). The effect of daunomycin on the nucleic acid metabolism and viability of HeLa cells has recently been reported from this laboratory (4). The studies reported in this paper, in which a mitotically synchronized culture was used, provide some information on cell viability and nucleic acid synthesis in HeLa cells following treatment with adriamycin and make possible a comparison of the biological activities of adriamycin and daunomycin.

MATERIALS AND METHODS

Experiments were carried out with HeLa S-3 cells in Eagle’s minimum essential medium supplemented with 15% fetal calf serum. Details of the cell culture procedure were described elsewhere (3). Tests for contamination of the HeLa cultures with mycoplasma were negative.

Synchronous cultures were obtained by selective collection and plating of mitotic cells (5). Labeling procedure, autoradiography, and determination of nucleic acids and protein have been described in detail elsewhere (6).

Cell counts were performed with a Model B Coulter counter. Plating for colony counts was carried out with 60-mm plastic Petri dishes. Control and adriamycin-treated plates prepared from trypsinized single cell suspensions or harvested mitotic cells (500 cells/plate) were incubated for 12 days at 37°. Colonies were fixed with methanol, stained with crystal violet, and counted after projection with a photographic enlarger. A colony containing more than 50 cells was considered to be reproductively intact.

RESULTS

Effect of Adriamycin on Nucleic Acid and Protein Synthesis. In view of the close structural similarity of adriamycin to daunomycin, which has been shown to be an effective inhibitor of nucleic acid synthesis in HeLa cells (4), an experiment was carried out in which asynchronously growing cells were exposed for various times to concentrations of adriamycin in the range of 0.01 to 1.0 μg/ml. Chart 1 shows that the rate of DNA and RNA synthesis was promptly reduced to about 20 and 50% of the control, respectively, within 1 hr after exposure to the drug (1.0 μg/ml). The rate of protein synthesis as measured by tritiated valine incorporation into the acid-insoluble fraction was not significantly reduced. Measurements of the total DNA, RNA, and protein content in replicate cultures showed a reduction in the amount of DNA and RNA following exposure of cells to the drug for a period of 12 hr, while that of protein is not appreciably reduced.

Effects of Adriamycin on Cell Division. For observation of the effect of adriamycin on cell multiplication, randomly growing cells were exposed to various concentrations of the drug for 32 hr. The control cells were growing exponentially, with a doubling time of about 18 hr, while the cell population exposed to the drug (0.01 μg/ml) increased slightly for 12 hr and then remained stationary (Chart 2).

Effects of Adriamycin on Cell Viability. The lethal effect of adriamycin in terms of colony formation of single cells was studied both in asynchronous and synchronous cultures of HeLa cells. The survival curves obtained with asynchronous cells showed less effect at short exposure times to the drug than at longer times (Chart 3). The lethal action of adriamycin...
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Chart 1. Effects of adriamycin on the incorporation of tritium-labeled thymidine, uridine, and valine into DNA, RNA, and protein, respectively. Twenty-two hr after the plating of $5 \times 10^5$ trypsinized cells, adriamycin was added and, at indicated times following the drug addition, 15-min pulses of thymidine-$^3$H (1.9 Ci/mmole, 1 $\mu$Ci/ml), 10-min pulses of uridine-$^3$H (20 Ci/mmole, 1 $\mu$Ci/ml) or 30-min pulses of valine-$^3$H (0.6 Ci/mmole, 3 $\mu$Ci/ml) were given to cells, which were then processed, and radioactivity was measured in a liquid scintillation counter. o, control (no drugs); •, adriamycin, 0.01 $\mu$g/ml; ◯, adriamycin, 0.1 $\mu$g/ml; ▲, adriamycin, 1.0 $\mu$g/ml.

Chart 2. Changes in the number of cells per plate following exposure of HeLa cells to adriamycin. o, control (no drug); •, adriamycin 0.01 $\mu$g/ml; ◯, adriamycin, 0.1 $\mu$g/ml; ▲, adriamycin, 1.0 $\mu$g/ml.

during the division cycle revealed that the drug was most toxic during S phase (Chart 4). A relatively high degree of synchrony and a normal rate of cell progression through the cell cycle were obtained, as demonstrated by the graph of the percentage of cells labeled with tritiated thymidine (Chart 4).

Comparative Study of Adriamycin and Daunomycin on Cell Viability. Chart 5 shows the relative dose response curves of asynchronously growing HeLa cells exposed for 1 hr to adriamycin or daunomycin. It is evident that daunomycin is significantly more effective in reducing the cell viability than adriamycin on a molar basis.

DISCUSSION

It is evident from the present experiments that adriamycin promptly inhibits the synthesis of DNA and RNA in HeLa cells (Chart 1). The rate of protein synthesis is not

Chart 3. Survival of asynchronously growing HeLa cells exposed to varying concentrations of adriamycin as a function of time of exposure. The drug was added to cells 20 hr after plating. Each point represents an average of 6 replicate plates. The plating efficiency of the control cells was 60%.

Chart 4. Survival of synchronously growing HeLa cells exposed to either adriamycin (0.3 $\mu$g/ml) or daunomycin (0.5 $\mu$g/ml) for 1 hr during the different phases of the division cycle. ◯-○, percentage of cells labeled with tritiated thymidine (10-min pulse) during the division cycle in the controls. AM, adriamycin; DM, daunomycin. The plating efficiency of the control cells was 55%. The data represent the average of 2 separate experiments.

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significant reduced, at least for the 1st 12 hr after exposure of cells to the drug. These results demonstrate that replacement of a hydrogen atom at the acetyl radical of the aglycone by a hydroxyl group does not appreciably alter the pattern of the inhibition of nucleic acid and protein synthesis. Although no in vitro studies on the interaction of the drug with DNA were carried out, as was the case with daunomycin (4), it may be conjectured that the basic inhibitory mechanisms of action of adriamycin and daunomycin are similar, on the basis of the rather close structural similarity between the 2 antibiotics.

The cell viability, defined here as the capacity of a single cell to grow out into a macroscopic clone, is reduced sharply following exposure of the asynchronous cell population to adriamycin (0.1 µg/ml) for a fractional period of the generation time (Chart 3). However, a comparison of the relative lethality of adriamycin with daunomycin shows that adriamycin is less toxic than daunomycin (Chart 5). The less toxic effect of adriamycin was also observed in in vivo animal systems (9). The lethal action of adriamycin during the division cycle shows that the drug is most effective in reducing the reproductive capacity of cells engaged in DNA synthesis (Chart 4). Again, the result with adriamycin is similar to that with daunomycin in our previous studies (4). The apparent mechanism for the differential lethal activity occurring during the division cycle is not known. Whether the accessibility to the drug of DNA in chromosomes may vary or whether the efficiency of the repair may change during the cycle cannot be determined without further experimentation.

Adriamycin has been shown, in several experimental animal systems, to have a higher therapeutic index than daunomycin (2, 7). Adriamycin is, in fact, less effective in killing HeLa cells than daunomycin on a molar basis. A recent in vivo comparative study of daunomycin and adriamycin shows that adriamycin is less cytotoxic than daunomycin to normal hematopoietic colony-forming cells in mice, although the lethal effect on the leukemic cells was more pronounced with adriamycin than with daunomycin (8).

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REFERENCES

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