Comparison of cancer chemotherapeutic agents in asynchronous and synchronous 9L cells

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Abstract

The cytotoxic activity of various chemotherapy agents was investigated in asynchronous populations of cultured 9L rat brain tumor cells, and as a function of their position in the cell cycle. Representative drugs from the classes of DNA-active agents, alkylating agents, spindle poisons, and antimetabolites were tested. The ability to induce cell lethality in asynchronous populations as a function of drug concentration varied for 1 hr pulse exposures. In order of decreasing cytotoxic activity, DHAQ was the most effective, followed by VCR, VDS, VBL, ADR, BCNU, cis-DDP, BLM, DBD, RZ, and HU. The effect of chemotherapy agents on synchronous 9L cells obtained by mitotic selection also varied with respect to the individual agent and was cell cycle-dependent. Survival age-responses ranged from being minimal to demonstrating significant fluctuations as a function of cell cycle position. For all agents except ADR and HU, the sensitivity of G1 phase was greater than S phase. RZ exhibited essentially a flat age-response. Comparison of the cell cycle age-responses of chemotherapeutic agents to those exhibited by the cytotoxic modalities of radiation and hyperthermia demonstrate several unique differences.

Abbreviations: ADR—Adriamycin, BCNU—1,3-Bis (2-Chloroethyl)-1-Nitrosourea, BLM—Bleomycin sulfate, cis-DDP—cis-Dichlorodiamineplatinum, DHAQ—Dihydroxy anthraquinone, DBD—Dibromodulcitol, HU—Hydroxyurea, RZ—Razoxane, VBL—Vindaseine Sulfate, VCR—Vincristine Sulfate, VDS—Vindesine Sulfate, HBSS—Hanks’ Balanced Salt Solution.

Introduction

In the evaluation of new drugs for their potential utilization as chemotherapeutic agents, determination of relative cytotoxic activities as well as the kinetics of cell killing is valuable [1]. Cytokinetic data obtained in cell cycle analysis may indicate dose and time manipulations of clinical scheduling for different combinations of drugs given simultaneously or in sequence. Knowledge of the cytotoxic efficacy at each stage of the cell cycle i.e., phase sensitivity, is important in predicting the percentage of cells in a particular tumor which might be affected. For example, tumors that have low-growth fractions, such as brain tumors, are most successfully treated with drugs that are cell cycle-non-specific, e.g., BCNU. Considering combination treatments, agents with different phase sensitivities could be paired in an attempt to achieve significant killing of all cells composing a tumor population. Attempts are being made to combine cytokinetic data with the knowledge of age-dependent responses so as to provide new approaches to combination therapy, e.g, accumulating a large population of cells in a specific cell cycle stage and then utilizing a second drug (or drug combination) for which the main killing effect is in that phase [1, 2]. For these reasons, evaluation of chemotherapeutic
agents should include studies not only in asynchronous but also synchronous populations of cells.

The 9L rat brain tumor system, used extensively in several laboratories over the last 10 years, has been established as a valid experimental model for brain tumor therapy. Cultured 9L cells have also been used to investigate mechanisms of action for several chemotherapy agents currently being evaluated in clinical trials of brain tumor therapy. These reports have described the response of 9L cells to 1,3-bis(2-chloroethyl)-1-nitrosourea [3-8], dihydrogalactitol [9], spirohydantoin mustard [10], and cis-dichlorodiamineplatinum [11]. Of these chemotherapy agents, only BCNU [4, 7, 8] and dihydrogalactitol [9] have been studied with respect to their ability to induce perturbations in the 9L cell cycle, and only one report provides information on relative cytotoxic activities as a function of cell cycle position [8].

The cell cycle age-response of 9L cells to radiation has been characterized [12, 13] and found to be unusual. Specifically, the fluctuations in survival across the interphase portion of the cell cycle were only minimal. In an effort to characterize further this brain tumor model system, cell cycle phase sensitivities were determined for several different classes of chemotherapeutic agents. Cytotoxicity studies were also performed in asynchronous cells.

Materials and methods

Cell culture techniques

9L/KC cells were maintained in exponential growth as monolayer cultures in McCoy’s Modified 5A medium supplemented with 10% fetal calf serum and (for experiments) antibiotics (penicillin, 100 units/ml; streptomycin, 100 μg/ml). All components were obtained from KC Biological, Lenexa, KS. Cultures grown in this medium at 37° in a humidified 5% CO₂ environment displayed a doubling time of 18 to 20 hours; with G1 = 9–10 hr, S = 5–6 hr, G2 = 2–3 hr, and M = 1 hr, as determined by labelling and mitotic indices measured every 2 hr as a function of time after incubating selected mitotic cells [13].

Colony formation assay

The ability of cells to survive following drug treatment was assayed by the retention of colony-forming ability. Plating efficiency was calculated as the number of colonies formed divided by the number of cells inoculated. Surviving fraction after treatment was calculated as the plating efficiency following treatment divided by the plating efficiency of control plates. Three replicate plates were inoculated per data point. Error bars associated with data points are standard deviations of the mean values.

Dose-response studies

For asynchronous dose response curves, cells from exponentially growing populations were trypsinized and subsequently plated into 60 mm diameter petri plates at cell concentrations such that between 50 and 200 colonies formed per plate after treatment. After cell inoculation, plates were incubated prior to treatment for at least 4 hr to allow cell attachment. Tests (data not shown) have indicated that this 4 hr period is also adequate in allowing complete recovery from the effects of trypsinization. Upon being treated, plates were returned to the incubator for 7–10 days. Plates were stained with methylene blue and the number of visible colonies of greater than 50 cells counted.