Spectrum of cell-cycle kinetics of alkylating agent Adolezesin in gynecological cancer cell lines: correlation with drug-induced cytotoxicity

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Summary. Adolezesin is an analog of CC-1065. These compounds are among the most potent alkylating agents known to date. Currently Adolezesin is undergoing phase I clinical trials at several cancer centers in the USA. While the cytotoxic effects of Adolezesin have been addressed elsewhere, its effects on cell-cycle kinetics have not been reported. Flow cytometry was performed on five human gynecological cancer cell lines: AN3, AE7, BG1, HEC1A, and SKUT1B. Exposure to Adolezesin (U73975, Upjohn Co.) was done at near confluency at 0, 0.1, 0.2, 0.5, 1 and 5x, with x = 10 pg/ml as reference concentration, for 90 min. Cell samples were taken by trypsinization at 0, 24, 48, 72, 96, and 168 h for flow cytometry. The ATP chemosensitivity assays were performed on the above cell lines to establish dose/response curves. Flow-cytometric analyses revealed that there was a spectrum of cell-cycle perturbations, which included biphasic S and G2 blocks, reverse dose-dependent G2 blocks, and a sequential relationship of S and G2 blocks. This study demonstrated that the cell kinetic response to Adolezesin depended on several variables such as cell lines, drug sensitivity, concentrations, and sampling time. Because of this multivariable dependence and the lack of correlation with cytotoxicity, it would be difficult to use cell kinetic perturbations to predict chemotherapeutic response.

Key words: Adolezesin – Cell kinetics – Cytotoxicity

Introduction

Adolezesin is a new synthetic derivative of CC1065. This family of compounds remains the most potent alkylating agents known to date (Hanka et al. 1978; Martin et al. 1980; Swenson et al. 1982). It was shown that CC1065 binds noncovalently to the minor groove of the DNA double helix in the adenine-thymidine-rich areas. This preferential binding allows the drug to reach its selected DNA targets and accounts for its extreme sensitivity (Reynolds et al. 1986; Dekoning et al. 1987). Among many synthetic derivatives, Adolezesin was chosen by Upjohn Company for further development because of the enhanced cytotoxicity and lack of lethal hepatotoxicity (Warpehoski 1989; Dekoning et al. 1987). Preliminary in vitro studies revealed that Adolezesin was approximately 105-106 times as potent as Adriamycin (Alberts et al. 1989). This drug is currently undergoing phase I clinical trials at various cancer centers in the USA.

Flow cytometry continues to play an essential role in studying cell-cycle kinetics. It has replaced traditional cytometric methods, such as thymidine labeling, and provided more information on the effects of cytotoxic agents on the cell cycle (Averette et al. 1970; Barlogie and Dreyfus 1978; Barlogie et al. 1983). Traditionally, it is believed that the same drug will exhibit the same patterns of cell kinetic response on various tumor cells because of having the same mechanism of action. Because of this belief, results of flow studies on a particular drug have often been assumed to be applicable to other cell lines and tumor systems. In fact, information gained from cell kinetic studies were often used to devise drug components for combination chemotherapy and the sequence in which drug delivery should occur (Barlogie et al. 1983; Tobey et al. 1979). It has also been suggested that the patterns of drug-induced cell kinetic effects of a patient's tumor sample could be used to predict his or her response to chemotherapy (Sevin et al. 1983, 1986; Sevin 1990; Tsurusawa et al. 1986). Cell kinetic studies of various cytotoxic agents have also allowed us to understand drug effects on the cell cycle. On the basis of this information, chemotherapeutic agents are often classified as cycle-specific or cycle-nonspecific drugs (Muss 1990; Thigpen 1987; Hill and Bellamy 1984). Among cycle-specific drugs, cytotoxic agents are further classified as primarily affecting the G1, S or G2 phases.

A review of the literature demonstrated that a majority of cell kinetic studies were restricted to a few drug con-
cells/ml was used to plate a 24-well tissue-culture flask in triplicate. Assays have been used successfully to study drug response (Sevin et al. 1988). Since the total amount of cellular ATP corresponds to cell number and cell mass, the ATP bioluminescence assay has been used to study drug response. Viable cell counting was carried out with a hemocytometer using the trypan blue exclusion technique. Cells were then lysed with nuclear isolating buffer, stained with propidium iodide and fluorescein isothiocyanate, and subjected to flow cytometry as previously described (Kingston et al. 1988).

**Materials and methods**

**Cell lines.** Five human gynecological cancer cell lines were used; the endometrial cell line AE7 was obtained from Dr. Satyaswaroop (Hershey, P.). This cell line was cultured from primary and untreated well-differentiated adenocarcinoma of the endometrium. Cell lines HEC1A, AN3, and SKUT1B were obtained from American Type Culture Collection. HEC1A was derived from an untreated moderately differentiated adenocarcinoma of the endometrium at 12th passage. Cell line AN3 was derived from a metastatic lymph node of a poorly differentiated adenocarcinoma of the endometrium of a patient previously treated with hormone. SKUT1B was isolated from a patient with poorly differentiated leiomyosarcoma, who had previously been treated with irradiation. Cell line BG1, generated from an untreated ovarian cancer patient, was kindly provided by Dr. Jeffrey Johnson, Bowman Gray University. All cell lines were grown in Eagle’s modified essential medium. The medium was prepared with 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2.5 μg/ml amphotericin B. Cells were incubated at 37°C with 5% carbon dioxide and 95% humidity. Medium was replaced every 3 days and cells were subcultured weekly following detachment with 0.25% trypsin/0.02% EDTA.

**Drugs.** Adolezesin was provided from the Cancer Research Unit, the Upjohn Company (Kalamazoo, Mich.). A reference concentration of 10 pg/ml or 0.02 nM was chosen on the basis of preliminary in vitro data and the manufacturer’s recommendation. A stock solution of 1 mg/ml Adolezesin in solvent dimethylacetamide was kept in the dark under refrigeration. It was dissolved in distilled water at appropriate concentrations immediately before use.

**ATP chemosensitivity assay.** Since the total amount of cellular ATP corresponds to cell number and cell mass, the ATP bioluminescence assay has been used successfully to study drug response (Sevin et al. 1988). ATP contents of treated cells relative to those of controls directly reflect surviving fractions. Briefly, a suspension of 20,000 cells/ml was used to plate a 24-well tissue-culture flask in triplicate. For each drug, besides the control wells, the following concentrations were used: 0.1, 0.2, 0.5, 1, 2, and 5 x with x equal to the reference concentration. Drug exposure was for 90 min at 24 h after plating. Dose/response curves were obtained on day 7 by extracting the ATP from the cells in situ with an equal volume of ice-cold 2% tri-chloroacetic acid. ATP bioluminescence was determined as previously described (Sevin et al. 1988).

**Flow cytometry.** A suspension of 10⁶ cells of the above cell lines was plated into 25-cm² flasks. Drug exposure was done at near confluency (approximately 72 h after plating) for the following concentrations: 0.0, 0.1, 0.2, 0.5, 1, and 5 x. Samples of controls and treated cells were taken by trypsinization 0, 24, 48, 72, 96, and 168 h after treatment. Viable cell counting was carried out with a hemocytometer using the trypan blue exclusion technique. Cells were then lysed with nuclear isolating buffer, stained with propidium iodide and fluorescein isothiocyanate, and subjected to flow cytometry as previously described (Kingston et al. 1988).

**Results**

Dose/response curves from the ATP chemosensitivity assays of five cell lines are presented in Fig. 1. These cell lines cover a whole range of drug response to Adolezesin. The most sensitive cell line was SKUT1B and the most resistant was BG1. Their corresponding mean IC₅₀ values (measured as a multiple of x) are given in order of decreasing sensitivity: 0.097 for SKUT1B, 0.14 for AN3, 0.36 for AE7, 0.53 for HEC1A, and 1.65 for BG1. Figure 2 shows cell-cycle perturbations of SKUT1B by Adolezesin. For doses less than 0.5 x, there was an insignificant cell kinetic response by SKUT1B cells, and its G₁, S, G₂ curves were indistinguishable form those of the control. Despite this lack of cell-cycle perturbations, correlation with the ATP chemosensitivity assays revealed surviving fractions of 60%, 9%, and 3% for Adolezesin doses 0.1, 0.2, and 0.5 x, respectively. Both viable cell counting and the ATP chemosensitivity assays confirmed significant cytotoxicity at these concentrations. Thus, there appeared a lack of correlation between the SKUT1B cell kinetic response and its cytotoxicity to Adolezesin doses of 0.1, 0.2, and 0.5 x. As the concentrations were increased to 1 and 5 x, one began to see S and G₂ blocks and G₁ depletion. These doses corresponded to surviving fractions of 2.2% and 0.9% respectively. One possible explanation for the discrepancy between cell kinetic response and cytotoxicity was tumor heterogeneity. As the sensitive tumor population died, a small but resistant subgroup of tumor cells survived and exhibited a cell-cycle response to Adolezesin only at high concentrations.

Cell-cycle effects of the AN3 cell line are shown in Fig. 3. This was the second most sensitive cell line with a mean IC₅₀ of 0.14 x. Of interest was a pattern of biphasic

![Fig. 1. Dose/response curves of AE7, ANE, BG1, HEC1A, and SKUT1B cell lines after a 90-min exposure to U73975. Doses were measured as multiples of x (reference concentration = 10 pg/ml).](image-url)