Gossypol, a Hyperthermic Sensitizer of HeLa Cells

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ABSTRACT

Gossypol, a polyphenolic aldehyde extracted from cotton plants, is a potent antifertility agent in humans. Since we have previously reported that several male antifertility agents including 5-thio-D-glucose and lonidamine demonstrate hyperthermic sensitizing effects in HeLa cells, we wished to determine whether gossypol also exhibits the hyperthermic sensitization. Gossypol was not cytotoxic up to 4 h at 37°C (10 µg/ml). When HeLa cells were exposed to gossypol at 41° and 42°C, significant potentiation of hyperthermia induced cytotoxicity was observed. The magnitude of the potentiation was dependent on the drug concentration, pH of the culture medium, glucose concentration, temperature, and duration of treatment. The hyperthermic sensitizing effect of gossypol was increased by an acidic pH and glucose deprivation. These data suggest that the sensitizing effect of the drug may be mediated through the lowering of cellular energy level by the inhibition of both glycolysis and oxidative phosphorylation.

INTRODUCTION

Gossypol is a polyphenolic compound occurring in the pigment glands of cotton plants. Gossypol first attracted attention as a potential male contraceptive as a result of extensive studies in China (1, 2). Although the mechanism of contraceptive action, the pharmacokinetics, and the toxicity of gossypol are not well defined, an intriguing feature of the antifertility action of gossypol is that the drug is selectively toxic to testicular tissues while having no observable effects on other tissues (1, 3-5). Recent cell culture studies showed that gossypol had antitumor effects against several tumor cell lines in culture (6-9). Tumor mitochondria-bound hexokinase (7). Since gossypol is a known antispermatogenic agent and interferes with the energy metabolism in the cell, we wished to determine whether gossypol also potentiates the cytotoxic effects of hyperthermia in HeLa cells. If the drug proved to be a hyperthermic sensitizing agent, we wished to define the culture conditions needed to maximize the effect.

MATERIALS AND METHODS

Experiments were carried out with HeLa S-3 cells grown in Eagle’s minimal essential medium supplemented with 10% fetal calf serum. Details of the cell culture procedures including the maintenance, the trypsinization, and the test for contamination with mycoplasma of cultures are described elsewhere (18, 20).

Plated monolayer cells were heated to within 0.05°C of the desired temperature by totally immersing plastic culture flasks in a water bath heated by a Haake Model 52 temperature circulator. Water bath temperatures were verified by a National Bureau of Standards thermometer.

The pH of the culture medium was adjusted by varying the CO2 content of the gas phase within the flasks. The buffering system of Eagle’s minimal essential medium consisted of 26 mEq NaHCO3 at 5% CO2 for a neutral pH of 7.4. To obtain a pH of 6.7, for example, the flasks were flushed with the gas mixtures containing 26% CO2. The pH of the culture medium was monitored throughout the treatment procedures by sealing a combination electrode (Altex combination pH electrode) in a treatment vessel and monitored with a temperature-compensated digital pH meter (Altex Model 3560).

The “glucose-deprived” medium was prepared by adding 10% dialyzed fetal calf serum to the culture medium without glucose obtained from Grand Island Biological Co. The dylated fetal calf serum contained less than 1 mg glucose/100 ml so that the final concentration of glucose in the glucose-deprived medium was less than 0.001 mg/ml.

Gossypol was purchased from Sigma Chemical Co. The compound was dissolved in dimethyl sulfoxide immediately prior to experiments. Dimethyl sulfoxide produced no enhancement of hyperthermic cytotoxicity with the range studied. The gas mixtures were purchased from Matheson Gas Products (East Rutherford, NJ).

RESULTS

Effect of Gossypol on Cell Multiplication. Preliminary experiments were carried out to determine the effect of gossypol on cell division. Exponentially growing HeLa cells were exposed to the drug for 72 h at 37°C. Control cells grew exponentially with a doubling time of approximately 19 h. Cells exposed to drug concentrations of 5, 10, and 20 µg/ml grew exponentially for the first 24-h period and then remained stationary. The growth curve of cells treated at a gossypol concentration of 2.5 µg/ml was not significantly different from the control for the initial 24-h incubation; however, at incubation times greater than 24 h, a lengthening of doubling time was observed (Chart 1). The growth rate of the cells exposed to 1.25 µg/ml was the same as that of the control cells.

Effect of Gossypol on Cell Survival at Elevated Temperature. Chart 2 shows the survival curves of cells as a function of...
Gossypol as Heat Sensitizer

**Chart 1.** Effect of gossypol on cell multiplication as a function of time at 37°C.

**Chart 2.** Effect of gossypol on heat sensitivity (°C) of cells under acidic or neutral pH conditions. The drug was present only during heating. Cell survival is expressed as a percentage of unheated control cells. The plating efficiency of control cells was 60-70% throughout the study.

**Chart 3.** Percentage of cell survival as a function of gossypol concentration for cells exposed at 42°C for 2 h under acidic or neutral pH conditions.

**Chart 4.** Effect of gossypol on survival of glucose-deprived cells exposed at 42°C under neutral pH.

**DISCUSSION**

The results of the present study demonstrate that gossypol is a hyperthermic sensitizer of HeLa cells. In particular, the hyperthermic sensitization becomes increasingly evident when heated exposure time under various pH conditions and drug concentrations. The survival curves of cells incubated at 37°C with gossypol, 5 and 10 μg/ml, for up to 4 h show no appreciable drug toxicity under acidic or neutral conditions. Since the drug concentrations and exposure time of 4 h did not show any detectable toxicity at 37°C, we selected these treatment conditions for the subsequent studies at elevated temperatures. It is apparent that the cytotoxic effect of hyperthermia on drug-treated cells was most pronounced under acidic conditions. The dose-dependent effect of the drug is clearly shown in Chart 3. For example, at 10 μg/ml and 2 h exposure at 42°C, cell survival is reduced to 1% under acidic condition. Heat treatment alone reduces cell survival to only 50% of the control.

**Effect of Gossypol on Glucose-deprived Cells.** Since gossypol has been reported to interfere with cellular energy metabolism (1, 2), experiments were performed to determine the influence of glucose on the cytotoxic effect of gossypol. Chart 4 shows the results of experiments under glucose-fed and -deprived conditions at pH 7.4. Incubation at 37°C with gossypol, 10 μg/ml, for 4 h under glucose deprivation produced no apparent cytotoxicity. However, when these cells were heated at 42°C for 4 h with gossypol, 10 μg/ml, a substantial increase in cell killing was seen in glucose-deprived cells. The survival of glucose-fed cells exposed to the same treatment was approximately 10% while that of glucose-deprived cells was about 1%. It appears that deprivation of glucose from cells can further enhance the cytotoxic effect of gossypol at elevated temperature.
cells are exposed to both gossypol under low pH and a glucose-deprived state (Charts 2, 3, and 4).

The degree of the increased cell kill produced by gossypol under acidic pH and progressive temperature elevations is impressive (Chart 2). Similar findings of the pH dependence for the drug’s effect were previously obtained with both lonidamine, an antifertility agent, and quercetin, a lactate transport inhibitor (18, 21). We have suggested in earlier publications that the sensitization of tumor cells to hyperthermia under low pH can be understood in the context of the energy equilibrium of the cell (18, 21–23). The accelerated metabolic demands of the hyperthermic state cannot be adequately met if the cell is deenergized accordingly. The rate of cell kill is increased under the conditions which produce an energy-depleted state. An acidic medium pH increases cellular energy demands, as the cell increases proton efflux through homeostatic mechanisms. A failure to restore the pH will further reduce the intracellular energy level by inhibiting glycolysis (24–26). In this context, it is conceivable that the treatment of cells by gossypol impairs cellular energy production through uncoupling of oxidative phosphorylation (10).

The hyperthermic potentiation produced by gossypol under glucose-deprived conditions is impressive even at a neutral medium pH (Chart 4). The mechanism involved may also be understood in the context of the cellular energy equilibrium. If gossypol uncouples oxidative phosphorylation, the deprivation of glucose in culture media of gossypol-treated cells results in a total blockade of ATP production rendering the cells extremely energy depleted and thereby sensitive to heat. We have obtained similar results in glucose-deprived cells with another mitochondrial-binding agent, rhodamine 123 (27). Gossypol has been shown to possess differential cytotoxic effects against various human tumor cells in culture. Among them, most sensitive to the drug were melanoma and colon carcinoma cells (7). If gossypol is found to have certain selective cytotoxic effects against tumor cells in vivo without undue normal tissue toxicity, it may have potential utility as a hyperthermic sensitizer, because the microenvironment of large tumors may favor the selective hyperthermic effects of gossypol by virtue of an acidic interstitial pH.

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