Selective Killing of Glucose and Oxygen-deprived HeLa Cells by Hyperthermia

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ABSTRACT

We previously have reported that glucose deprivation specifically enhances cell killing by hyperthermia in the absence of oxygen. The present studies were carried out to evaluate the interplay of glucose and oxygen on cell killing by hyperthermia (37 to 42°) for up to 4 hr under varying concentrations of glucose (0 to 1 mg/ml) and oxygen (0 to 21%). pH 7.4 was maintained in all trials. In the absence of oxygen, enhanced cell killing at 40 and 42° was seen with glucose concentrations below 0.1 and 0.25 mg/ml of media, respectively. In the absence of glucose, enhanced killing was seen when oxygen concentrations were below 1 and 2% at 40 and 42°, respectively. The radiosensitivity of the cells was influenced only by the oxygen concentration during irradiation and not by glucose. These results indicate that oxygen and glucose are important modifying factors of cell lethality by hyperthermia.

INTRODUCTION

Cell culture studies have shown that cancer cells in general are more sensitive to heat than are normal cells (1, 2, 5, 6, 8, 17). However, at present, little is understood about the mechanisms of action of hyperthermia at cell level. Cell culture studies to date have identified several important factors that influence the thermal response of cells to hyperthermia: cell cycle phase; cellular growth stage; cellular acidity; and ambient oxygen concentration (3, 4, 7, 13-15, 19-22, 26).

Recently, it was reported that hypoxic cells were as sensitive to heat as oxic cells (4). Since hypoxic cells must derive the energy needed to survive via anaerobic glycolytic pathway and are heat sensitive, we carried out a series of studies using an inhibitor of glycolysis (5-thio-D-glucose) on HeLa S-3 cells under oxic and hypoxic conditions (11, 16, 23-25). The results showed that the combined treatment of hyperthermia with the glycolytic inhibitor enhanced the killing of hypoxic cells selectively at elevated temperatures but not of oxic cells. In the present report, we attempted to determine the importance of glucose and oxygen as a modifying factor for hyperthermic cellular damage. Preliminary findings of this study were reported elsewhere (10).

MATERIALS AND METHODS

Experiments were carried out with HeLa S-3 cells grown in Eagle’s MEM supplemented with 10% FCS. Details of cell culture procedure including the maintenance, the trypsinization, and the test for contamination of cultures with Mycoplasma have been described elsewhere (9, 10, 12). No antifungal agent was used throughout this study.

Cell survival was assayed by the colony-forming ability of single-plated cells to obtain quantitative dose-survival curves. Details of cloning experiments including colony count were described elsewhere (10, 15).

The method used to obtain oxic and hypoxic cells was essentially the same as described by Gerweck et al. (4). In order to obtain hypoxic cells, Belco T-15 glass tissue culture flasks (approximate growth area, 15 sq cm) containing approximately 7 x 10^6 exponentially growing HeLa cells were flushed with water-saturated gas mixtures containing 0 to 2% oxygen, 5% CO₂, and balance of N₂ at a flow rate of 150 ml/min for 15 min. The gas mixtures were prepared, analyzed, and certified by Matheson (East Rutherford, N. J.). Oxic cells were similarly prepared except that the flasks were flushed with air containing 5% CO₂ and approximately 21% oxygen.

The “glucose-deprived” medium was prepared by adding 10% dialyzed FCS to the MEM without glucose obtained from Grand Island Biological Co. The dialyzed FCS contained less than 1 mg glucose per 100 ml or 0.01 mg/ml so that the final concentration of glucose in the “glucose-deprived” medium was less than 0.1 mg glucose per 100 ml or 0.001 mg per ml. Glucose concentration of culture media was varied by adding an appropriate amount of glucose to the “glucose-deprived” medium.

The cells were heated to within ±0.05° of the desired temperature by completely immersing the culture flasks in a water bath heated by a Haake Model 52 temperature circulator. The temperatures were verified by a National Bureau of Standards thermometer (15).

Irradiation was performed with a 0.667-MeV Gammacell 40 (¹³⁷Cs) of Atomic Energy of Canada, Ltd., with a dose rate of 115 rad/min at room temperature.

The cells were kept under the various concentrations of glucose and oxygen only during the treatment period.

Following heat treatment or irradiation, the cells were trypsinized and replated in 60-mm Petri dishes. Enough cells were plated to form 100 to 200 colonies after treatment and 10 to 12 days incubation.

The buffer system of MEM consists of 26 mM NaHCO₃ and 5% CO₂ (pH 7.4). All the gas mixtures used in the study contained 5% CO₂, and the pH of culture media was monitored throughout the experiments by a combination electrode (Corn ing combination pH electrode).

RESULTS

Effect of Glucose on Oxic and Hypoxic Cell Survival at Elevated Temperatures. The effect of glucose concentration...
in medium on cells in the presence or absence of oxygen was studied at 37–42°C (Chart 1). The cells were exposed to medium containing varying amounts of glucose during heat treatment. The cell survival of oxygenated cells under a complete glucose-deprived medium was minimally altered at temperatures from 37 to 42°C. In contrast, when hypoxic cells were heated in glucose-deprived medium at 40 to 42°C, a substantial enhancement of cell killing was obtained relative to that of hypoxic cells under glucose-replenished medium. Chart 2 illustrates the details of cell survival response as a function of glucose concentration under oxic and hypoxic conditions. The data clearly show that the reduction of glucose content in culture media enhances the hyperthermic killing of cells under hypoxic conditions. At 40°C, 0.1 mg glucose per ml was sufficient to protect the cells from the enhanced killing, and at 42°C, 0.25 mg glucose per ml was needed to protect the cells.

Effect of Oxygen Concentration on the Survival of Cells during Hyperthermia in the Presence or Absence of Glucose. To determine the significance of oxygen content in culture media on the survival of cells following hyperthermia in the presence or absence of glucose, the cells were exposed to elevated temperatures with various oxygen concentrations in the media. The results of studies are shown in Chart 3. As with the effect of glucose deprivation on hypoxic cells, only those cells heated in the absence of both glucose and oxygen showed enhanced killing. There was no enhancement of cell killing of hypoxic cells heated in the presence of glucose. Chart 4 shows the details of the interplay of the survival of cells in the presence and absence of glucose as a function of ambient oxygen concentration at temperatures of 37 to 42°C for a 2-hr period. It is again clear that the absence of oxygen in culture media enhances the hyperthermic killing of cells heated in the glucose-deprived media only. At 40°C, 1% oxygen was sufficient to protect the glucose-deprived cells from heat while at 42°C, 2% oxygen was required for the same protection.

Radiosensitivity of Oxic and Hypoxic Cells Irradiated in the Presence or Absence of Glucose. Because the heat sensitivity of hypoxic cells was altered by the presence or absence of glucose during heating, the effect of glucose on the radiosensitivity of hypoxic cells was also studied. Both the glucose-fed and glucose-deprived cells were irradiated with a

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**Chart 1.** Survival of HeLa cells incubated with various concentrations of glucose in culture media at 37 to 42°C for 0 to 4 hr in the presence or absence of oxygen. Cell survival is expressed as a percentage of unheated controls. The plating efficiency of control cells was 60 to 70%. Glucose concentrations: ▲, 0 mg/ml; □, 0.05 mg/ml; ○, 0.1 mg/ml; ◆, 1.0 mg/ml.

**Chart 2.** Survival of oxic and hypoxic HeLa cells exposed to different temperatures for 2 hr with various concentrations of glucose in culture media. Cell survival is expressed as a percentage of the unheated control cells. The plating efficiency of control cells was 60 to 70%. ○, 37°C; ▲, 40°C; ◆, 41°C; ▲, 42°C.
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in the culture medium during heating greatly enhances the thermal sensitivity of cells. The presence of either glucose or oxygen completely abrogates the selective killing of cells at elevated temperatures.

Chart 6 summarizes the quantitative relationship of glucose and oxygen concentration at 42° for 2 hr. Also shown is the dependency of radiosensitivity on oxygen concentration but not on glucose (Chart 6b).

We have previously reported that the treatment of hypoxic cells with 5-thio-D-glucose, an inhibitor of glucose metabolism, selectively enhanced cell killing at elevated temperatures (11, 16). The similar findings obtained with glucose-deprived culture media indicate that the basic mechanism of 5-thio-D-glucose for enhanced hyperthermic killing of hypoxic cells would be mediated via a reduction of glucose availability to the hypoxic cells.

DISCUSSION

We report here that deprivation of both glucose and oxygen

Chart 5. Survival of glucose-fed and glucose-deprived HeLa cells irradiated in the various concentrations of oxygen. Cell survival is expressed as a percentage of unirradiated control cells. The plating efficiency of control cells was 60 to 70%. Oxygen concentrations: △, 0%; △, 0.5%; ●, 1.0%; ○, 2.1%.

137Cs γ source at room temperature under the various ambient oxygen concentrations. The survival curves summarized in Chart 5 show that the absence of glucose during irradiation did not alter the radiosensitivity of cells. The survival curves obtained with the glucose-fed and glucose-deprived cells were identical, and as expected, only the variation in oxygen concentration influenced the cell survival. At 10% survival level, the oxygen enhancement ratio of oxic (21% oxygen) and hypoxic (0% oxygen) cells was 3.3. At 0.5% oxygen concentration, the oxygen enhancement ratio was reduced to 1.8.
Additional factors that might influence the thermosensitivity of cells to the magnitude of the present findings would include cell age response function and ambient pH of culture medium. A pH of the culture medium below 7.0 is known to enhance the thermosensitivity of cells (3). However, there was not a selective sensitization of thermal injury between cells heated under oxic and hypoxic conditions by low pH (20). It would be interesting to find out whether cells under hypoxia and glucose-deprived medium could further be sensitized by lowering pH during heat treatment. As with radiation, thermosensitivity also markedly fluctuates during the cell cycle with the S phase being the most heat sensitive. It seems unlikely that the selective thermal sensitization of glucose-deprived hypoxic cells in this study could have resulted from the redistribution of cell populations into the sensitive S phase, particularly since we observed a prompt reduction of cell survival within 1 hr of heat treatment. Nevertheless, information is obviously needed to predict cell progression under our experimental conditions.

Foci of hypoxic cell populations are considered to be an integral part of rapidly growing solid tumors (18). Cells under chronic hypoxic conditions may become metabolically inactive and exist in a resting state, yet they may retain clonogenic properties for some time before they die. According to the data reported here, the changes in cellular environment due to the deprivation of both oxygen and glucose can render these radioresistant populations of tumor cells extremely sensitive to heat.

It is difficult at present to speculate on the biochemical mechanism of action of glucose deprivation on cells heated under hypoxic conditions. The present finding that glucose is necessary for the survival of cells heated under hypoxic conditions suggests that hyperthermia might alter the glucose metabolism of these cells. Whether this enhanced lethal effect can be further modified by either metabolic inhibitors of glycolysis or glycolytic intermediary metabolites is under investigation.

REFERENCES


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