Response of Human Hematopoietic Precursor Cells (CFUc) to Hyperthermia and Radiation

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ABSTRACT

Currently, whole-body and local hyperthermia is being evaluated in clinical studies as a potential method of cancer treatment. Since the hyperthermic sensitivity of normal human bone marrow cells is not known, we have studied the in vitro response of these cells to two anticancer modalities when administered alone or in combination. Cell survival following various treatment schedules was determined by colony formation of bone marrow cells (CFUc) in soft agar suspensions. Within the survival range studied, a thermal tolerant plateau on the cell survival was not observed for temperatures of 42°C or less. However, toleration induction could not be ruled out. In addition, when hyperthermia (42.5°C for 1 hr) and radiation (100 rads) were sequenced, the human CFUc survival remained the same regardless of whether the radiation was administered before, during, or after the hyperthermic exposure. Under our experimental conditions, we found the human CFUc to be more radiosensitive (D0 = 84 rads) than what has been reported previously. The radiation survival response of human CFUc was similar for cells irradiated either in vitro or in vivo. The possible clinical implications for these data are discussed.

INTRODUCTION

The limiting toxicity of many antineoplastic regimens is bone marrow suppression. Thus, it would be beneficial to have the capability of studying bone marrow cells through controlled laboratory investigations. Using modifications of a technique to study murine hematopoietic precursor cells (19), human bone marrow cells may also be cloned (24). The individual cells that give rise to colonies consisting of mature granulocytes, monocytes, and macrophages have been termed CFUc.1 The CFUc is believed to be a committed precursor cell derived from the hematopoietic pluripotent stem cell (25). The murine CFUc has been used to study the effects of hyperthermia (22), radiotherapy (32), and chemotherapy (18). However, few data are available describing the cytotoxic effects of hyperthermia (22), radiotherapy (28, 32, 35) and chemotherapy (18) on the human CFUc. We are not familiar with any study that has investigated the effects of hyperthermia on the human CFUc.

In the last few years, there have been several laboratory investigations suggesting a role for hyperthermia in cancer therapy (8, 12, 13, 20). Hyperthermia at temperatures in excess of 41°C has been shown to be cytotoxic to mammalian cells (29, 30). Unlike radiation, hyperthermia has been shown to have an enhanced cytotoxic effect on cells that are hypoxic (9) at low pH (10, 11) and are actively synthesizing DNA (S phase) (34). In addition, hyperthermia potentiates radiation damage by inhibiting sublethal (1) and potentially lethal (16) radiation repair. Several in vitro studies have shown that simultaneous hyperthermia and radiation exposure are synergistic with respect to cell killing (29, 31).

In vivo studies have suggested that there may be a differential response of normal tissues and tumors to hyperthermia. One report using embryonic tissues and adult fibroblasts as normal tissue controls has suggested that their neoplastic counterparts may be more thermosensitive (12). There are few data available to attribute this definitely to individual cellular sensitivities. However, differences in cellular response to hyperthermia have been attributed to environmental factors such as hypoxia (9), acidic pH (23), and tumor blood flow (3). Based on these experimental findings, several clinical trials are presently underway utilizing either local field hyperthermia (14, 17), or whole-body hyperthermia (5, 6).

Since the response of normal human bone marrow to hyperthermia and combinations of hyperthermia and radiation is not known, we investigated the response of normal human hematopoietic precursor cells (CFUc) to these cytotoxic agents.

MATERIALS AND METHODS

Bone marrow aspirates from the posterior iliac crest were collected from both normal volunteers and selected cancer patients without known bone marrow metastases. Appropriate informed consent forms were obtained from all patients. Separation of nucleated bone marrow cells and RBC was achieved by mixing marrow aspirates (1 to 3 ml), HBSS without calcium and magnesium, and 6% dextran (M, 2.23 x 106) in normal 0.9% NaCl solution to the ratio of 1:1:0.5 and incubating for 15 min in a 37°C incubator. The supernatant was collected, then washed twice in HBSS containing 10% fetal calf serum, resuspended with parafilm. The pH of the agar suspension during all treatments was maintained at 43°C. The addition of 43°C agar solution resulted in only a minor elevated temperature was on the order of 5 min. The final agar concentration in the medium (room temperature) and a 1.29% solution of Difco's special acid agarose was achieved.
maintained at 7.3. For each experiment, the same number of cells (8 x 10^6) were plated into a number of T-25 flasks. The flasks were then placed in a 37° incubator for 3 hr. A 3-hr preincubation period at 37° was arbitrarily chosen to allow cells to recover from the dextran separation and plating. Following the 3-hr incubation, the various experimental protocols were conducted, and then the flasks were returned to the incubator for colony formation.

After 7 to 12 days of incubation, colonies containing 50 or more cells were counted using an inverted phase microscope. The plating efficiency consistently ranged between 0.01 and 0.05%. Therefore, for each dose-response curve, the number of colonies counted for control flasks ranged between 80 and 400. Each experimental point was determined from the average of colony counts from 2 to 3 flasks. Variation in colony counts from flask to flask for a given point was usually not more than 10%. Individual experiments were repeated several times, each time using a different patient's bone marrow aspirate. Where experiments are repeated several times, the data from each individual experiment are presented. Survival curves were drawn by eye using all the data for a given condition.

One study involved the comparison of the radiation response of bone marrow cells irradiated in vitro and in vivo. A previously untreated patient with Stage I seminoma undergoing pelvic irradiation agreed to this experiment. The first anterior, posterior radiation field encompassed the left iliac crest. Ten min prior to this treatment, a bone marrow aspirate was taken from the designated irradiation field. Part of this sample served as a control, and the remainder was used to determine an X-ray survival curve for in vitro irradiation. Following the anterior radiation exposure (calculated dose to iliac crest, 55 rads), a second aspirate was taken from the same site. A third aspirate was taken after the posterior radiation exposure (total dose after third aspirate, 55 + 95 rads = 150 rads). The entire procedure was completed in approximately 25 min. In all cases, each aspirate was immediately mixed with equal volumes of HBSS and maintained at 37° for 3 hr, after which they were plated following the procedure described previously.

Cell samples were irradiated using a 6 MeV photon beam from a Mevatron VI linear accelerator. The dose rate was 200 rads/min. Dosimetry was carried out using a Baldwin Farmer ion chamber connected to a Keithly electrometer system having a direct National Bureau of Standards calibration. For all irradiations, the flasks were submerged in a circulating lucite water bath (37°) positioned beneath the beam. Full electron equilibrium was ensured for all irradiations.

Hyperthermia treatment consisted of immersing the sealed flasks in precision temperature-controlled water baths (Lauda Model B-1; Brinkmann Instrument, Inc., Westbury, N. Y.) capable of maintaining temperature within ±0.05°. All thermometers (mercury in glass) used were calibrated against a certified thermometer, the calibration of which was traceable to the National Bureau of Standards. The cells were maintained at 37° at all times, except when they were heated to the treatment temperatures. The flasks were transferred rapidly from 37° baths to the hyperthermia baths for treatment. The half-time for temperature transition for the 4-ml agar suspension in a T-25 flask was approximately 30 sec as determined by measurements with a thermometer surface probe (Yellow Springs Instrument Co., Yellow Springs, Ohio) positioned at middepth in the agar suspension.

RESULTS

The hyperthermic response of human CFUc is shown in Charts 1 and 2. Two or 3 individual experiments were conducted for each curve. In general, the hyperthermic response observed for human CFUc is similar to that reported for other mammalian cells (26). The major difference, however, is the apparent lack of a thermal tolerant plateau at 42°, at least within the survival range studied. Little cell killing was observed for the first 8 to 10 hr of exposure at 41°. A gradual decline in survival for treatment times between 10 and 24 hr was observed.

The X-ray survival curve for human CFUc is illustrated on Chart 3. Each symbol represents survival curve data from one of 6 different donors. The survival curve is characterized by a D0 value of 84 rads and an extrapolation number, n = 1.18. Each bone marrow sample was irradiated at 37° 3 hr after plating the cells in complete medium. The multiplicity at the time of irradiation was assumed to be 1.0.

Two experiments were conducted in order to determine the survival of CFUc using various sequences of hyperthermia and radiation. The cells were treated with a heat dose of 42.5° for 1 hr combined with a radiation dose of 100 rads given before, during, or after heating as shown in Chart 4. Qualitatively, the 2 experiments are in good agreement, with similar cell survival for all sequences of treatment.

The effect of heat treatment for 10 hr at 41°, 1 hr at 42.5°, and 0.5 hr at 43° on the cellular radiosensitivity is shown in Chart 5. Heat doses were chosen to give approximately the same level of survival. One line has been drawn through all of
Survival of Human CFUc to Heat and X-Rays

When compared to other mammalian cells, the human CFUc exhibited both similar and differing responses to hyperthermia. The major differences involved: (a) the apparent absence of a thermal tolerant plateau during continuous heating at temperatures of 42° or lower and (b) little difference in survival response when hyperthermia and radiation treatments were sequenced. At temperatures of 43° and less, thermal tolerance (as evidenced by a plateau in the survival curve) in several other established cell lines and murine systems usually develops after 3 to 4 hr of heating. Even though a pronounced plateau in the survival curve was not observed for human CFUc within the survival range studied, thermoduric induction cannot be ruled out. The fact that there is a substantial difference in survival at 42° and 42.5° may suggest that thermoduric at 42° had developed and would possibly be expressed as a thermoduric plateau at lower survival levels. The CFUc assay used in these studies does not permit accurate survival estimates below 10⁻² surviving fraction. Future experiments involving fractionated heat exposures may clarify whether human CFUc exhibit thermoduric. Cells exposed

![Chart 3. In vitro X-ray survival curve for human CFUc. Each symbol represents survival data from a bone marrow sample of a different patient.](image)

![Chart 4. Survival of human CFUc following various sequences between heat and radiation. Radiation (100 rads) was administered either before, during, or after a 1-hr hyperthermic exposure at 42.5°.](image)

![Chart 5. Survival of human CFUc subjected to hyperthermia and radiation. Cells were first exposed to heat at various temperatures and durations (○, 41° for 10 hr; □, 42.5° for 1 hr; △, 43° for 0.5 hr) to give equal survival levels. The cells were then irradiated at 37° 5 min after termination of the heat treatments. ---, the X-ray survival curve redrawn from Chart 3.](image)

![Chart 6. Bone marrow cells from one patient were irradiated in vivo and in vitro and assayed for survival in vitro. The dashed curve is the X-ray response of CFUc irradiated in vitro redrawn from Chart 3.](image)

DISCUSSION

Since bone marrow suppression is a major toxicity of many antineoplastic regimens, the response of hematopoietic precursor cells to these therapies is of clinical interest. The CFUc assay does not measure the "true" pluripotent hematopoietic stem cell. However, there is considerable evidence that suggests that the CFUc and the pluripotent stem cell parallel each other with respect to quantity (25). There are also data from canine studies which suggest that there is a relationship between the quantity of CFUc and hematopoietic reconstitutive capabilities (21).
to 41°C for a prolonged duration exhibit a biphasic survival curve. During the first few hr, there was little cell killing. After 10 to 12 hr of exposure, exponential cell killing was observed. Read and Bedford (27) have also demonstrated a similar response for Chinese hamster ovary cells exposed for prolonged periods at 41°C. These investigators have attributed the inflection in the survival curve to cells progressing into S phase, a thermosensitive stage of the cell cycle. Data from [H]thymidine suicide experiments using the murine spleen colony assay have indicated that, under normal control mechanisms, a large proportion of hematopoietic precursor cells remain in the G0 phase (25). They can, however, be induced to proliferate by a variety of stimuli (25). Thus, it may be reasonable to speculate that the inflection at 10 hr in the 41°C CFUc survival curve may be due to movement of the cells into the S phase.

Sequencing of hyperthermia and radiation exposure may be of clinical importance. Enhanced cell killing of mammalian cells has been demonstrated when radiation is delivered simultaneously with hyperthermia or when the radiation is delivered after the heat treatment to cells maintained at acidic pH. For human CFUc, cell survival remained the same regardless of whether the radiation was administered before, during, or after the hyperthermia exposure. We have not investigated cell survival in regard to sequencing for higher temperatures and increased radiation doses.

Wide field irradiation encompassing a major portion of the bone marrow such as used in the treatment of lymphomas may result in significant myelosuppression. This has led investigators to examine the radiosensitivity of bone marrow cells. D0 values between 127 and 159 rads have been reported for human CFUc (4, 15, 32, 35), while for mouse CFUc, the values reported are lower (D0 = 115 rads) (33). For all of these studies, the extrapolation number (n) has ranged between 1.0 and 1.6. Under our experimental conditions, we found the human CFUc to be more radiosensitive (D0 = 84 rads) than what has been reported previously. This discrepancy may be due to variations in culture conditions during and after irradiation. All of our radiation cell survival curves were carried out in complete medium at 37°C, whereas some of the studies mentioned above irradiated the cells at 4°C (4, 35) or at room temperature in 0.9% NaCl solution (32). It has been demonstrated for several cell systems that postirradiation conditions, such as incubation in 0.9% NaCl solutions or incubation at reduced temperatures, can result in the repair of potentially lethal damage (2). These differences in technique may explain our results of increased radiosensitivity of human CFUc.

Twenty years ago, a major issue in cellular radiation biology was whether the radiosensitivity of mammalian cells in culture was the same as their radiosensitivity in vivo. Till and McCulloch (33), in 1961, demonstrated that mouse bone marrow cells have a similar radiosensitivity when irradiated in vitro or in vivo. To our knowledge, this has not been shown for any human tissues. Therefore, we thought it of interest to examine this issue with the human CFUc. Because of technical limitations, only one patient was studied. For the 2 radiation doses used, there was good correlation of cell survival for CFUc irradiated either in vivo or in vitro. In addition, this experiment was interesting from the standpoint of the feasibility of obtaining quantitative cell survival data from patients.

We have attempted to demonstrate the response of normal human hematopoietic progenitor cells to 2 agents used for cancer therapy. Normal tissue toxicity is a major concern in cancer therapy and, in particular, with combined modality therapy. Ultimately, it would be beneficial to determine the clinical correlation of myelosuppression with the in vitro CFUc survival response to various anticancer agents. If such correlations were possible, the in vitro CFUc assay might be useful in predicting toxicities induced by antineoplastic agents prior to their introduction into clinical studies.

REFERENCES


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