Heat Sensitivity, Thermotolerance, and Profile of Protein Synthesis of Human Bone Marrow Progenitors

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ABSTRACT

Hyperthermic sensitivity, kinetics of thermotolerance induction and decay, and profile of heat shock protein synthesis were studied in human granulocyte-macrophage progenitors and nucleated bone marrow cells, respectively. The Do of the heat survival curves of human granulocyte-macrophage colony-forming cells at 43°C, 44°C, and 45°C were 23, 12, and 5 min, respectively.

The kinetics of thermotolerance induction was measured with a triggering dose of 44°C/20 min, 45°C/10 min, 41°C/2 h, and 42°C/1 and 4 h. The heat shock protein synthesis was measured after a triggering dose of 44°C/20 min or 45°C/10 min. Synthesis of M, 70,000 and 87,000 heat shock proteins in the total nucleated bone marrow cells was evident at least for 8 h after the initial heating.

INTRODUCTION

WBH is currently being used in the treatment of refractory leukemias and lymphomas (1-3). Studies on the response of murine and human bone marrow progenitors to hyperthermia have recently been performed by our laboratory and other investigators (4-11). Studies on the effect of heat on murine bone marrow progenitors have shown CFU-GM and CFU-M to be more heat resistant than the erythroid progenitors, CFU-E and BFU-E. CFU-S, the myeloid stem cells, seem to be the most heat sensitive (12, 13).

Current interest in the hyperthermic sensitivity of blood cell progenitors results from the fact that some leukemias have been shown to be more sensitive to heat than normal bone marrow stem cells (12-14). Robin et al. (14) have shown that the AKR murine leukemia is more sensitive to heat than CFU-S, while Symond et al. (12) found that L1210 leukemias growing in bone marrow were more sensitive to heat than CFU-S. Furthermore, studies with human myeloid leukemias have shown them to be more sensitive than normal human granulocyte-macrophage progenitors (Ref. 15; Footnote 3). Hyperthermia could be a useful modality in the treatment of leukemias if, indeed, there is a differential sensitivity between the leukemic cells and normal bone marrow stem cells. More studies are needed in this area, especially regarding human bone marrow stem cells and human leukemias.

One of the important aspects of hyperthermia treatment is the ability of cells to develop transient resistance to subsequent heat challenges (16-19). This transient resistance is called thermotolerance and may severely limit the efficacy of subsequent heat treatments. The phenomenon of thermotolerance has been studied in a variety of tumors and normal tissues (17). Thermotolerance in blood cell progenitors has been recently studied by several investigators (4, 5, 7). Murine bone marrow progenitors have been shown to develop thermotolerance. However, thermotolerance decays faster than in other cell lines or tumor tissues (17). Bromer et al. (7) showed that human granulocyte-macrophage progenitors did not develop chronic thermal resistance when heated at 41°C or 42°C continuously for 24 h.

In this paper, the kinetics of the development and the decay of thermotolerance and heat shock protein synthesis has been investigated in human granulocyte-macrophage progenitors and various nucleated bone marrow stem cells, respectively.

MATERIALS AND METHODS

Bone Marrow Preparation. Bone marrow aspirates from the posterior iliac crest were collected from normal donors. Bone marrow aspirates were diluted 1 ml:2.5 ml of phosphate-buffered saline and 2.3 ml of bone marrow/phosphate-buffered saline mixture:1 ml of Ficoll-Hypaque (Pharmacia). The cell suspension was then centrifuged at room temperature at 400 x g for 40 min. The top layer containing granulocytes and monocytes was withdrawn and diluted in MEM plus 15% FCS, and an aliquot was counted by hemocytometer.

In Vitro Heating and Incubation. Nucleated cells were suspended in MEM (Gibco Laboratories, Grand Island, NY) plus 15% FCS (Gibco) at approximately 2 x 10^6 cells/ml in a 15-ml centrifuge tube, flushed with 5% CO2/95% air to maintain the pH at 7.4, and immersed and heated in a circulating water bath (Haake DB; Fisher Scientific, Pittsburgh, PA). Temperature was maintained within ±0.02°C. Temperature was measured with calibrated mercury-in-glass thermometers.

For thermotolerance development and decay studies, cells were incubated in IMDM (Gibco) plus 20% FCS.

Cell Survival Assays. After appropriate treatment the cell suspension was centrifuged, and 0.2 ml of IMDM were added to the pellet. The cells were vortexed gently, and 1.8 ml of the following medium was then added to each tube: 0.4 ml of FCS; 0.02 ml of penicillin and streptomycin (10,000 μg/ml); 0.49 ml of IMDM; 0.79% methylcellulose; and 0.1 ml of conditioned medium which was obtained by growing the human bladder carcinoma cell line 5637 in IMDM plus 2% FCS at the density of 5 x 10^4 cells/ml for 7 days. The human bladder carcinoma cell line 5637 has been reported to produce a colony-stimulating factor for granulocyte and macrophages (20). One ml of the cell suspension was placed in 30-mm Falcon Petri dishes and was incubated at 37°C in 98% humidity for 10 to 14 days. Colonies with >40 cells were counted with an inverted microscope at x10 magnification (21).

Plating efficiency varied between 0.05 and 0.07% for unbeated controls which were immediately plated following Ficoll-Hypaque separation (50 to 70 colonies/10^4 nucleated cells). The plating efficiency did not change during the 24- and 48-h incubation at 37°C. All experiments were performed at least 2 times. Unless otherwise indicated, a representative experiment is shown. The error bars are the mean ± SD. The Do values were derived by using the method of least-square regression analysis of the straight-line portion of the survival curves.

Labeling and Electrophoresis. Approximately 3 x 10^6 cells were suspended in methionine-free MEM and labeled for 2 h at 37°C with [35S]methionine (80 μCi/ml; specific activity, >600 Ci/mmol; Amer-
THERMOTOLERANCE OF HUMAN CFU-GM

Fig. 1. Heat response of human CFU-GM. Total nucleated cells heated in MEM plus 15% FCS at 41-45°C. Cells were then plated in methylcellulose medium for colony formation. Bars, SD.

Fig. 2. Thermotolerance in human CFU-GM receiving a priming heat dose of 44°C. Nucleated cells were given a priming dose of 44°C/20 min, and after 2-, 4-, 6-, 24-, and 48-h incubation at 37°C, the cell suspension was heated at 44°C for various times.

Results of HSP synthesis of total nucleated cell. Figs. 6 and 7 show the kinetics of HSP synthesis in the total nucleated cells after a priming dose of 44°C/20 min. HSP synthesis was measured at 0, 2, 4, 6, 24, and 48 h after incubation at 37°C. Synthesis was measured at 0, 2, 4, 6, 24, and 48 h after incubation at 37°C. Synthesis of HSPs was evident up to 8 h following the initial heat dose.

DISCUSSION

There is increasing evidence that some leukemic cells may be more sensitive to cytotoxic effects of heat than normal bone.
Fig. 4. Thermotolerance in human CFU-GM receiving a priming heat dose of 41°C and 42°C. Nucleated cells were given a priming heat dose of 41°C/2 h or 42°C/1 h and 4 h. After 0-, 2-, 4-, 24-, and 48-h incubation at 37°C, the cell suspension was heated at 45°C for various times.

Fig. 5. Kinetics of the development and decay of thermotolerance in human CFU-GM. Nucleated cells were given a priming heat dose of 44°C/20 min or 45°C/10 min (left) and 41°C/2 h or 42°C/1 h and 4 h (right); following incubation at 37°C up to 48 h, cells received a test dose of 44°C/60 or 45°C/40 min. The increase in survival and gradual return to control value were used as a measure of the development of thermotolerance.

marrow progenitors (11–15). As a result WBH is being tested for the treatment of refractory leukemias and lymphomas. Since bone marrow is generally the limiting organ in systemic therapy, its response to hyperthermia may be critical to the success of WBH. In this study we have examined the heat sensitivity and kinetics of thermotolerance development and decay of normal granulocyte-macrophages.

The results show that human CFU-GM are more resistant to heat than their murine counterpart (4). The only other study reporting on heat sensitivity and thermotolerance development of human CFU-GM is that of Bromer et al. (7). They showed no chronic thermotolerance development when bone marrow cells were heated at 41°C or 42°C for 24 h. To investigate whether CFU-GM were tolerant during 41°C and 42°C heating, the following experiments were performed. Cells were heated at 41°C/2 h or 42°C/1 or 4 h (Fig. 4). The cells were then challenged at various times with 45°C. The results indicated that CFU-GM were able to develop thermotolerance at 41°C or 42°C.

Thermotolerance was also observed when the priming dose of heat was 44°C or 45°C. Thermotolerance in all cases was maximum by about 6 h and decayed by 24 to 48 h. These results are similar to that of murine CFU-GM (4), for which thermostolerance decayed within 48 h irrespective of the severity of the heat treatment. Thermotolerance of murine and human CFU-GM decays relatively faster than other mammalian tumor or cell lines previously studied (17, 23). The reason for the relatively fast decay of thermostolerance in murine and human CFU-GM may be due to cells differentiating out of the “CFU-GM”
pared to other tissues may make the fractionated whole-body hyperthermia a useful addition to a preparative regimen during bone marrow transplantation of leukemias and lymphomas. Fractionated whole-body hyperthermia may spare normal tissues due to thermotolerance while destroying the residual regenerating leukemic cells.

REFERENCES


Fig. 7. Autoradiogram of sodium dodecyl sulfate-polyacrylamide slab gel of [35S]methionine-labeled proteins of human bone marrow cells after 45°C/10 min of heat shock. The enhanced synthesis of M, 70,000 HSP is evident. C, unheated control cells. 0, 2, 4, 6, 24, and 48 h are recovery time (37°C) in h following the priming heat dose. Molecular weights (× 10^{-3}) are shown at left. A, actin (M, 43,000).
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