Effect of Hyperthermia on the Survival of Normal Human Peripheral Blood Mononuclear Cells

Shyam S. Agarwal, Edward J. Katz, and Lawrence A. Loeb

The Department of Medicine, King George's Medical College, Lucknow, India 226003 [S. S. A.]; Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111 [E. J. K.]; and Joseph Gottstein Memorial Cancer Research Laboratory, Department of Pathology SM-30, University of Washington Medical School SM-30, Seattle, Washington 98195 [L. A. L.]

ABSTRACT

Human peripheral blood mononuclear cells from normal healthy volunteers were exposed to elevated temperatures of 41–43°C for up to 6 hr. Thereafter, the cells were stimulated with phytohemagglutinin in vitro in order to measure indirectly the surviving fraction. DNA replication in heated cells in response to phytohemagglutinin was found to be a sensitive indicator of thermal injury. Exposure to even 40°C for 2 hr lowered thymidine incorporation at early time points after phytohemagglutinin stimulation, but the cells were able to recover from thermal injury after exposure for up to 4 hr at 42°C. At 43°C, exposure for even 1 to 2 hr caused irreversible damage. The changes in thymidine incorporation were due not to changes in endogenous nucleotide pools since parallel changes were observed in DNA polymerase activity. Thus, the heat sensitivity of normal human lymphocytes could be a limiting factor for use of hyperthermia as an adjunct to radiotherapy and chemotherapy of human cancer.

INTRODUCTION

The role of hyperthermia in the treatment of cancer, as an adjunct to radiotherapy and possibly chemotherapy, is increasingly recognized (5, 6, 13, 14, 20). Both local and systemic heat have been shown to be effective in the treatment of experimental and human tumors. However, the experience with human cancers has not been firmly established.

Studies on several established cell lines have shown that cell survival decreases above 42°C. Arrhenius plots based on these survival curves indicate a break in the inactivation at 43°C (16). In general, temperatures in excess of 43°C are used for treatment of cancer. However, the safety of this temperature to normal tissues has not been adequately documented. In our earlier studies, we have observed that the upper limit of temperature that allows optimum response of human peripheral blood mononuclear cells to PHA is 40°C (3, 23). At 41°C and 42°C, DNA replication in PHA-stimulated lymphocytes is markedly reduced. Mononuclear cells are key cells in the immune response, and immunoregulation could play a significant role in the control of cancer. If hyperthermia is to be used widely in the treatment of cancer, it is important that the effects of exposure to elevated temperatures on the survival and proliferative response of lymphocytes be investigated. In this paper, we show that normal human mononuclear cells may recover from damage resultant from exposure to heat at 42°C for up to 4 hr but not from the effect of exposure to 43°C for as little as 1 to 2 hr. This heat sensitivity of normal human lymphocytes may be a limiting factor for the use of hyperthermia in the treatment of human cancer if temperatures in excess of 42°C are used.

MATERIALS AND METHODS

Cells. Human peripheral blood was obtained from normal healthy volunteers. Mononuclear cells, including T-cells, non-T-cells, and monocytes (macrophages), were isolated by sedimenting the diluted blood on Ficoll-Hypaque gradients. The cells were washed three times with Roswell Park Memorial Institute Medium 1640, and the final cell concentration was adjusted to 1 x 10⁶ cells/ml. Culture medium consisted of Roswell Park Memorial Institute Medium 1640 with 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer supplemented with 1 mM L-glutamine, penicillin (100 units/ml), streptomycin (100 µg/ml), and 20% fetal calf serum. One-mL cell aliquots in 10- x 125-mm Falcon plastic culture tubes were heated by incubating in a water bath at 40°C, 41°C, 42°C, and 43°C for varying lengths of time up to 6 hr. The cultures were then maintained in humidified CO₂ incubators at 37°C for 1 hr prior to addition of PHA (PHA-M; Grand Island Biological Company, Grand Island, N. Y.). Fifty µl of recommended dilution of PHA were added to each culture, and the cultures were maintained at 37°C for the indicated time.

Trypan Blue Dye Exclusion Test. The cell suspension (0.1 ml) was mixed with an equal volume of 0.4% trypan blue dye. The cells were left at room temperature for 5 min and then evaluated in both chambers of a hemocytometer. At least 200 cells were counted and, in case of discrepancy by more than 10% between the 2 chambers, the procedure was repeated. Cells were designated as viable if they excluded the dye and were not stained.

Measurement of [³H]Thymidine Incorporation and DNA Polymerase Activity. Two hr prior to termination of cultures, [³H]thymidine (2.5 µCi; 6.7 Ci/mmol) was added to each culture, and the cultures were returned to the incubator at 37°C. At the end of 2 hr, the incorporation of thymidine was stopped by adding 5 ml of ice-cold 0.15 M KCl to each culture. The cells were pelleted at 2500 rpm x 10 min in an RC-2B refrigerated Sorvall centrifuge and were washed twice with ice-cold 0.15 M KCl. The cell pellet was then frozen and thawed, and the cell lysate was used for the assay of DNA polymerase. For the latter, [α-³²P]dATP was used as one of the 4 deoxynucleotide triphosphate substrates, activated calf thymus DNA as added template, and Mg⁡⁺ as the metal activator. After incubation for 1 hr at 37°C, radioactivity incorporated in an acid-insoluble product was quantitated. [³H]thymidine incorporation was taken as the rate of thymidine incorporated into DNA by PHA-stimulated lymphocytes in culture, and [³²P]dATP incorporation was taken as a measure of DNA polymerase activity in vitro. Details, control, and analysis of the product of the reaction have been described previously (2).

RESULTS

Measurement of Cell Viability by Trypan Blue Dye Exclusion Test. Human peripheral blood mononuclear cells were exposed...
Effect of Heat on Lymphocytes

Human peripheral blood mononuclear cells were cultured as described under "Materials and Methods." One-ml aliquots containing 10^6 cells/ml in medium with 20% fetal calf serum were heated at 41°-43° for different lengths of time. Immediately at the end of each heating period, the viability of the cells was determined by trypan blue dye exclusion method, as described under "Materials and Methods." After incubation at 37°, PHA was added to the cultures. [3H]Thymidine incorporation and DNA polymerase activity were measured as described under "Materials and Methods." O, 37° (average, 5 cultures); •, 40° (average, 3 cultures); ■, 41° (average, 3 cultures); △, 42° (average, 3 cultures); ⧮, 43° (average, 3 cultures). Points, mean; bars, S.D. The standard deviations at 28 and 46 hr were too small to be presented. Thymidine incorporation was significantly lower in 28 hr in cultures heated at 40°, 41°, 42°, and 43°, the values being 60%, 69%, 85%, and 92%, respectively, compared to the 37° control of 83%. DNA polymerase activity was significantly lower only at 42° and 43°.

### Table 1

Viability of cells measured by trypan blue dye exclusion test

<table>
<thead>
<tr>
<th>Incubation (hr)</th>
<th>% of viable cells after heating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37°</td>
</tr>
<tr>
<td>0.5</td>
<td>NT^a</td>
</tr>
<tr>
<td>1</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>4</td>
<td>97</td>
</tr>
<tr>
<td>6</td>
<td>98</td>
</tr>
</tbody>
</table>

^a NT, not tested.
DISCUSSION

A role for hyperthermia in the treatment of cancer is increasingly recognized. However, no guidelines exist for choosing an optimal schedule for heat therapy for the treatment of human cancers. Thermal sensitivity of normal human tissues is one of the major considerations in planning schedules for the treatment of cancer with heat. The results presented in this paper show that exposure of normal lymphocytes for up to 4 hr at 42° does not cause any permanent damage, while exposure at 43° for even 1 to 2 hr completely and irreversibly diminishes the proliferative potential of these cells. This indicates that the efficacy of heat treatment protocols, where temperatures in excess of 42° are used, should be reevaluated with respect to immune functions of lymphocytes in treated individuals. It also needs to be determined whether local heat treatment, without isolation of circulation, affects circulating lymphocytes.

Measurement of response to PHA after exposure of lymphocytes to noxious stimuli may provide a sensitive method for monitoring damage to these cells (1). We find that while 90% of the mononuclear cells exposed to 43° for 2 hr were capable of excluding trypan blue, they were not able to undergo DNA synthesis after stimulation with PHA. Although trypan blue dye exclusion test is a simple and rapid method for testing viability of cells (9, 11, 19), discrepancies between this test and other biological parameters of viability have been reported (13). The trypan blue dye test does not accurately reflect the thermal damage to lymphocytes (11, 15, 16, 21). Apparently the effect of heat on those cell membrane functions that determine dye exclusion is indirect or delayed. This is supported by the fact that, immediately after exposure to heat, only 25% of the cells heated at 43° for 3 hr were not able to exclude the trypan blue dye, while after additional incubation at 37° for 12 hr 75% of the heated cells were stained. A similar delayed effect of heat on cell membrane function has been reported by Lin et al. (17) and Schrek (22), though at higher temperatures.

The mechanisms by which elevated temperatures affect normal and malignant cells are unknown. The synergism between heat and radiation has been attributed to a heat-mediated inhibition of the repair of sublethal damage to DNA caused by radiation (4). Hyperthermia has been shown in most (8, 10) but not all studies (6, 20) to decrease the rate of both single- and double-stranded DNA repair (8). It is to be noted that eukaryotic DNA polymerase β, which has been hypothesized to function in DNA repair, is genetically heat sensitive (12). Activity of DNA polymerase β is diminished in vitro by 50% after incubation for 15 min at 42°. It is conceivable that the potential synergism of heat and radiation may be mediated by selective inactivation of DNA polymerase β. This is in accord with recent studies on the correlations between inactivation of DNA polymerase β (when whole cells are heated) and radiosensitization and heat-induced cell killing (24).

Our preliminary studies on the effect of elevated temperature on DNA repair show that exposure of Somer cell line lymphoblastoid cells to 42° for 4 hr effectively inhibits the rejoining of X-ray induced single-strand breaks. If these in vitro results can be extended and confirmed by clinical trials, exposure to 42° for 4 hr prior to X-irradiation may be a useful protocol for heat therapy as an adjunct to radiotherapy. The safety of 42° is supported by the observation that elevations of body temperature up to 41.8° under experimental conditions (7), and up to 41-42° with various natural infections and disorders of heat regulation, are well tolerated. Our in vitro studies reported here show that damage to normal human cells at 42° for 4 hr is reversible. We have chosen peripheral blood lymphocytes for these studies since lymphocytes are key cells for immune responses, and an intact immune system could be of considerable advantage in the overall control of cancer.

ACKNOWLEDGMENTS

We are thankful to Dr. Sudhir Gupta for his helpful suggestions and comments. Also, the technical assistance of M. Tuffner is gratefully acknowledged.

REFERENCES

Effect of Hyperthermia on the Survival of Normal Human Peripheral Blood Mononuclear Cells

Shyam S. Agarwal, Edward J. Katz and Lawrence A. Loeb


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/43/7/3124

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.