Hyperthermia and Thermal Tolerance in Normal and Ataxia Telangiectasia
Human Cell Strains

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ABSTRACT

Three normal human fibroblast strains, two human ataxia telangiectasia heterozygote cell strains, and two human ataxia telangiectasia homozygote cell strains were studied for their thermal responses between 41.0 and 46.0°. The heat sensitivities of all cell strains were comparable, and all cell strains were relatively heat resistant compared to Chinese hamster cells. Both normal and ataxia telangiectasia human cells developed thermal tolerance during heating at temperatures less than or equal to 43° and during incubation at 37° after acute heating at 45.0°. For survival measured down to the 5 to 10% level, heat survival curves for all seven human cell strains lacked shoulders, indicating the inability of such cells to accumulate sublethal heat damage. Analysis of the cell survival curve data by the method of Arhenius showed that the thermal inactivation energies for human cells were 127 and 230 kcal/mol above and below the break at 43.5°, respectively, and are about the same as for Chinese hamster cells and other animal cells, implying similar mechanisms of heat inactivation.

INTRODUCTION

Many different cultured cell lines have been used to study the effect of hyperthermia on mammalian cells (3, 7, 15, 20), and such studies have provided results leading to fundamental concepts that are germane to the use of hyperthermia in the clinic for cancer therapy (4, 11). Many experimental clinical programs are in progress, and reports indicate that tumors respond to hyperthermia and that normal tissue has good tolerance to heating (1, 6, 9, 10, 12, 13). The resistant nature of normal tissues may be a result of tissue properties, such as good vascularity and cooling by circulation, or because of an intrinsic heat resistance of the human cell. Almost all studies on the effects of hyperthermia on cultured cells have been done with animal cells, and thermal sensitivity can vary extensively among different cell lines (20). Thus, more data on the thermal response of human cells are needed to determine the response of human cells in culture and compare the results to those obtained for animal cells.

This study was carried out to characterize the thermal response of human normal fibroblasts and AT1 cells over a temperature range of 41.0–46.0°. A preliminary report on thermal sensitivity at 42.0 and 45.0° has been written (19). Both AT and normal cells were examined. Patients with AT are very radiosensitive (16), making radiotherapy in such patients difficult. Hyperthermia may provide an alternate means for cancer therapy in such patients.

MATERIALS AND METHODS

The cells used in all experiments were human fibroblasts. The AT5-BI cells were kindly donated by Dr. H. Nagasawa (Harvard University, Boston, Mass.), the GM38 cells by Dr. M. C. Patterson (Atomic Energy of Canada, Chalk River, Ontario, Canada), and all other cells by Dr. J. B. Mitchell (NIH, Bethesda, Md.). The GM38 cells were cultured in F-12 medium containing 15% fetal calf serum; the AT5 cells were cultured in F-12: Dulebecco’s modified medium (1:1) containing 5% fetal calf serum and 10% calf serum; and all other cells were cultured in F-12 medium containing 5% fetal calf serum and 10% calf serum. The GM38, GM3440, and AG1522 are normal cell strains; the AG3057 and AG3059 are AT heterozygote cell strains; and the GM2052 and AT5-BI are AT homozygote cell strains. Cells were subcultured once a week, and the cell passage numbers were between 12 and 20. The plating efficiencies for the AG1522, GM3440, GM38, AG3057, AG3059, GM2052, and AT5-BI cell strains were 7 to 10, 3 to 5, 1 to 2, 1.5 to 2.5, 1 to 2, and 4 to 10%, respectively. Cell cycle times for the normal human fibroblast ranged from 22 to 30 hr while AT cells had longer cycle times.

For experiments, exponentially growing cells were trypsinized with 0.1% trypsin (VMF highly purified trypsin, 20 units/ml) for 5 to 10 min, counted, and then plated in T75 flasks containing 10 ml of medium. The flasks were incubated 18 to 20 hr before experiments were started. No feeder cells were used in these experiments. For heating, the flasks were sealed with wax and immersed into temperature-controlled water baths. Heating was terminated by immersing the flask in a 37° water bath. The temperature maintained in the water baths was constant and uniform to ±0.02° (S.D.) by the use of a Tempunit Model TU14 thermal regulator and additional stirring pumps. The half-time for temperature equilibration in a T75 flask containing 10 ml of medium was about 30 sec. After heating and equilibration to 37°, the flasks were placed in a 37° incubator. After 7 days, 10 ml of fresh medium were added to each flask, and then each flask was further incubated. The flasks containing normal cells were fixed and stained for colony counting after 14 to 20 days, and the flasks containing AT cells were fixed and stained after 20 to 24 days. The V79 cells used as a comparison in these experiments were cultured in basal medium containing 13% fetal calf serum and grown and treated in T25 flasks. A complete description is given elsewhere (18). Survival was assayed by counting colonies that contained approximately 50 cells or more. For each data point, 3 T75 flasks were scored, and the S.E. is shown when greater than the data point symbol. Experiments were repeated 2 to 4 times, and all curves were fitted by eye.

RESULTS

The survival results for heating normal human cells from 41.0–46.0° are shown in Charts 1 and 2. For all temperatures tested, no shoulders were observed on the heat survival curves, indicating a lack of ability to accumulate sublethal heat damage. However, the possibility of the development of shoulders for heating to lower survival levels at the high temperatures above which thermal tolerance occurred cannot be ruled out. Thermal tolerance occurred for prolonged heating at temperatures less than or equal to 43.0°. At 43.0°, thermal tolerance started after about 2 to 3 hr of heating but was lost for heating times longer than...
Thermal Killing and Tolerance in Normal and AT Human Cells

Tolerance was evident for continuous heating at temperatures equal to or greater than 44.0° (Chart 2). The dashed curve in Chart 2 represents the heat survival of V79 cells during 45.0° treatment, and it is clear that these cells are much more heat sensitive than are the human cells heated at 45.0 or 46.0°.

Heat survival of several human AT cell strains is shown in Charts 3 and 4. During heating at 42.0°, all the AT cell strains developed thermal tolerance after about 4 to 6 hr of heating. The AT heterozygotes and homozygotes are approximately equally heat sensitive, and this heat sensitivity is comparable to the heat sensitivity of the normal human cell strains. Like the heat survival curves for the normal cell strains, the curves for the AT cells also had no shoulders, demonstrating a lack of ability to accumulate sublethal heat damage.

The survival data from Charts 1 to 4 are plotted in the
Arrhenius form (5) in Chart 5, and data for V79 cells (18) have been included for comparison. When the inverse of the survival curve slope is plotted versus the inverse of the absolute heating temperature, the slope of the resulting curve represents the thermal inactivation energy (5). The curves for the V79 cells and the normal human cells possess a break at about 43.5°C, and the thermal inactivation energies for human and V79 cells are 127 and 139 kcal/mol above 43.5°C and 230 and 233 kcal/mol below 43.5°C, respectively. For AT cells, it is not clear where the curve should lie because of the paucity and scatter in the data from the 4 cell strains. The data for human cell strains show that all, normal as well as AT, cell strains are approximately equally heat sensitive and, at all temperatures, are more heat resistant than are V79 cells.

All human cell strains tested also developed thermal tolerance after acute heating at 45.0°C as depicted in Chart 6, A and B. Cells were given an initial heat treatment at 45.0°C for 10 min and then incubated at 37°C to permit the development of thermal tolerance for a second heat treatment at 45.0°C. During the first 2- to 4-hr incubation at 37°C, thermal resistance increased rapidly, and, at longer incubation intervals, up to 11 to 14 hr, only relatively small further increases in thermal resistance were observed. The survival ratios for survival after the 11-hr incubation divided by survival after no incubation were 2.7 for the

\[ \text{AG1522 and GM3440 normal cell strains, 2.8 for the AG3057 and AG3059 AT heterozygote cell strains, and 5.2 for the AT5-BI A homozygote cell strain. Thermal tolerance did not develop to as large an extent as in rodent cell lines, but this may be due to the fact that human cells were heat resistant, and consequently, the initial heat treatment was less damaging.} \]

**DISCUSSION**

The characteristics of heat inactivation of human cells appear similar to those of animal cells. Cell killing at temperatures less than or equal to 43.0°C results in a rapid decrease in survival followed by the onset of thermal tolerance after about 4 hr of heating, as also observed in animal cells (3, 4, 20). At higher heating temperatures, thermal tolerance was not observed. Further, human cells develop thermal tolerance during incubation at 37°C after acute heating at 45.0°C, which is also observed in animal cells (3, 4, 8). The Arrhenius analysis shows that the thermal inactivation energies are approximately the same for human fibroblasts and Chinese hamster V79 fibroblasts and comparable to the values reported in the literature (4, 11).

The Arrhenius plot shows that the human fibroblasts and AT cells are approximately equally heat sensitive and that they were more resistant than were V79 cells and many cell lines reported in the literature (4, 11, 20). This intrinsic resistant nature of the human cells may, in part, account for the small response of normal tissue during clinical hyperthermia (1, 6, 9, 10, 12, 13).

The thermal responses of normal human fibroblasts and AT homozygote and heterozygote cell strains are comparable both
qualitatively and quantitatively. Thus, hyperthermia might be a less risky and difficult method than radiation for cancer therapy in such patients. Since normal and AT human cell strains have comparable heat sensitivities, the mechanisms responsible for increased radiosensitivity in AT cells (14, 17, 19) do not play a role in thermal cell inactivation, thus implying that the natures of heat and radiation damage are different. Our experiments that are in progress (data to be published) show that thermal enhancement of radiosensitivity is larger for the radiosensitive AT cells than for normal cells.

None of the heat survival curves obtained from normal or AT human cell strains possesses shoulders for survival down to the 5 and 10% level, while shoulders are commonly observed on heat survival curves of animal cells (3, 4, 7, 15, 20). These results agree with earlier data on human HeLa cells (11) and demonstrate that such cells lack the ability to accumulate sublethal heat damage. The absence of shoulders on the heat survival curves is a characteristic of both normal and AT human cells and is thus not related to the defects in AT cells that result in increased radiosensitivity. In asynchronous cell populations, cells at various stages of the cell cycle have different heat sensitivity, and this may have an effect on survival curve shoulders. This possibility cannot be ruled out for the human cells in these experiments.

Since human cells respond to hyperthermia in a qualitatively similar manner as animal cells, the development of thermal tolerance during heating or between fractionated heat treatments should be an important consideration in clinical hyperthermia.

REFERENCES

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