Development of Thermotolerance in a Human Melanoma Xenograft

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ABSTRACT

The kinetics of thermotolerance in a human malignant melanoma grown in athymic mice (BALB/cnu/nu/BOM) was studied. Local hyperthermia was given by immersing the tumor-bearing leg of the mice into a thermostatically regulated water bath. The tumors were exposed to a priming heat dose of 42.5°C for 30 min and, at different fractionation intervals, to graded heat doses at 42.5°C. Tumor volumetric tripling time, i.e., the time from the day the first treatment was given to the day the tumor volumes had reached 3 times the initial volumes, was used as measure of response. The thermotolerance ratio, i.e., the ratio of the slopes of the dose-response curves (tumor volumetric tripling time versus heating time) for preheated tumors and single-heated tumors, was used as the measure of thermotolerance. Thermotolerance developed rapidly; the thermotolerance ratio reached a maximum of 4.9 ± 0.3 (S.E.) at 16 hr and then decayed slowly to 1.1 ± 0.1 at 168 hr (7 days). Implications of the present results for treatment of cancer patients with hyperthermia are discussed, and it is concluded that treatment protocols probably should not prescribe more than one hyperthermic treatment per week.

INTRODUCTION

There is considerable experimental and clinical evidence that hyperthermia, either used alone or in combination with radiation or cytotoxic drugs, may be beneficial in the treatment of some types of cancer (3, 7, 13, 47). Since radiation therapy usually is given in many fractions, it may be inferred that fractionated treatment also will be used with hyperthermia. Hyperthermic treatments are known to induce a transient resistance to subsequent heat exposure, a phenomenon called thermotolerance (6, 17). Consequently, design of optimum fractionation regimens requires information on the kinetics of development and decay of thermotolerance in the tumor and the surrounding normal tissue.

Thermotolerance of cells in culture has been demonstrated as a reduction in the slope of the heat survival curves with or without a change in the shoulder (8, 9, 15, 18, 19, 36, 37). The magnitude and the kinetics of the thermotolerance were found to depend on the priming heat dose (8, 18, 33, 38) and the culture conditions (10, 11, 36, 37). Thermotolerance was obtained in some murine normal tissues (4, 12, 16, 21, 23, 28-30, 44, 50) and in a few rodent tumors (25, 34, 39, 51, 52) has also been studied. Different end points were used, and the experimental design in some of the studies did not allow a differentiation between thermotolerance and repair of sublethal heat damage. Nevertheless, the results from the in vitro experiments were qualitatively similar to those from the in vivo experiments. Thermotolerance developed gradually to a maximum, usually within 24 hr, followed by a slow decay for 2 to 4 days.

The purpose of the present work was to study the kinetics of thermotolerance in a human tumor grown in athymic mice. A malignant melanoma (E. E.) (45, 46) was chosen, since this type of tumor is used currently in investigations of clinical effects of hyperthermia (1, 24, 26, 27, 43). The results are compared with those reported for rodent normal and neoplastic tissues, and implications for clinical treatment of cancer with hyperthermia are discussed.

MATERIALS AND METHODS

Mice and Tumor. Female BALB/cnu/nu/BOM mice, kept under specific-pathogen-free conditions, were used. The malignant melanoma (E. E.) was derived originally from a lymph node metastasis in the left axilla of a 62-year-old man. Tumor tissue was transplanted directly into athymic mice without previous adaptation to in vitro culture conditions. Histologically, the metastasis was composed of melanin-poor atypical nevus cells growing in large spheres. Cells and nuclei varied greatly in size and shape, and numerous mitoses were seen. The melanoma was grown serially in athymic mice by implanting fragments, approximately 2 x 2 x 2 mm in size, s.c. into the flanks of recipient animals. Passages 42 to 49 of the melanoma in athymic mice were used in the present work. Small tumor fragments were implanted s.c. in the lower part of the thigh of the right hind leg of the mice. The tumors were exposed to heat when they had reached a volume of 200 to 500 cu mm. The diameters of the tumors were measured with calipers. Experiments showed that the variation in the measurements was considerably larger for the depth than for the length or the width of the tumors and that the depth usually was not significantly different from the width. Thus, 2,2-circular diameters (length and width) were recorded routinely, and the tumor volumes were calculated as V = 0.5 x ab², where a and b are the longest and the shortest diameters, respectively. Since the skin around the tumors was thin, the measured tumor diameters were not corrected for the skin thickness. Light and electron microscopic examinations showed that the histological appearance of the xenograft in passage 49 was similar to that of the metastasis in the donor patient. Hyperthermic Treatment. The tumors were heated by immersing the tumor-bearing leg of the mice into a water bath. Nonanesthetized mice were placed in Perspex mouse holders and immobilized by a piston. A hole was cut in the holders through which the leg with the tumor protruded. The leg was loosely fixed with tape without impairing the blood flow. The temperature of the water bath was thermostatically kept at 42.7°C. The temperature in the tumors, measured with a needle thermocouple probe (diameter, 0.7 mm) connected to an electric universal thermometer (Elilab, type TE 3-S; Elilab Instruments A/S, Copenhagen, Denmark), was 42.5 ± 0.1°C (S.E.) a few min after the tumors were immersed in the water bath and varied within this range during prolonged treatments. Experiments showed that the temperature readings did not vary significantly with the position of the probe in a tumor or among individual tumors.

Evaluation and Statistical Analysis. Tumor volumetric tripling time, i.e., the time from the day the first treatment was given to the day the tumor volumes had reached 3 times the initial volumes, was used as measure of response. The correlation between tumor volumetric tripling
time and initial tumor volume was for all treatment groups studied by linear regression analysis, and a t test was applied to determine whether the slope was significantly different from zero. A f test was also applied to investigate whether the volume-doubling time during regrowth varied significantly among the different groups. The S.E.s presented for tumor volumetric tripling time include the propagated errors starting with the volume measurements.

RESULTS

Statistical analysis showed that the response to a given treatment was independent of the initial tumor volume, which ranged from 200 to 500 cu mm. Mean normalized tumor volume following some heat treatments is shown in Chart 1. Heating resulted in inhibition of tumor growth before the tumors again grew exponentially. The exponential part of the growth curves was nearly parallel for the controls and the heated tumors, irrespective of heating time. Statistical analysis confirmed that the volume-doubling times during this regrowth period were not significantly different for the various treatment groups. Chart 1a refers to tumors given single heat treatments and shows that the tumor volumetric tripling time increased with increasing heating time. Chart 1b refers to tumors given 2 equal heat treatments of 30 min with different fractionation intervals. The tumor volumetric tripling time was always shorter for tumors exposed to 2 fractions of 30 min than for tumors given a single treatment of 60 min.

In order to study the effect of split-dose heat treatments in more detail, tumors were exposed to a first fraction of 30 min and a second fraction of 60 min at different fractionation intervals (Chart 2). Tumor volumetric tripling time decreased with increasing fractionation intervals up to about 16 hr. Further increase in the fractionation interval up to 168 hr resulted in a gradual increase in the tumor volumetric tripling time. The dashed lines in Chart 2 indicate tumor volumetric tripling time ± S.E. after a single heat treatment of 60 min, i.e., the same treatment as the second fraction in the split-dose experiments. Assuming complete repair of the heat damage caused by the first fraction and unaltered response to the second fraction, volumetric tripling times in the range between the dashed lines are to be expected. However, for fractionation intervals of 4 to 72 hr, the tumor volumetric tripling times were shorter than that indicated by the upper dashed line. Consequently, the first heat treatment had induced resistance to the second heat treatment; i.e., thermotolerance developed in the time intervals between the 2 fractions.

Thermotolerance was studied quantitatively by giving tumors a first heat treatment of 30 min and then, after different fractionation intervals, second graded heat treatments (Chart 3). The dose-response curve (tumor volumetric tripling time versus heating time) for tumors exposed to single heat doses showed a small initial shoulder. However, the data for tumors given split-dose treatments did not indicate dose-response curves with similar shoulders. Thus, linear dose-response curves, forced through the point for the tumor volumetric tripling time after a single treatment of 30 min, were fitted to the data by regression analysis. The slopes of the curves, which give a measure of the heat sensitivities of the tumor, are presented in Table 1. The
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Chart 3. Tumor volumetric tripling time as a function of total heating time for a human melanoma xenograft given 2 treatments at 42.5°C. The first treatment lasted 30 min, and the second lasted 15, 30, 45, 60, or 90 min. The fractionation intervals were 0 (•), 4 (○), 8 (△), and 16 (□) hr (a), and 24 (■), 48 (◆), 72 (◇), 120 (●), and 168 (×) hr (b). Each point is based on 15 to 20 tumors and represents mean values. S.E.s for the split-dose treatments were always of about the same order as those in Chart 2 and as those for the single treatments (indicated by bars), but they were omitted in the chart for clarity. The slopes of the curves were calculated by linear regression analysis and are presented in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Fractionation interval (hr)</th>
<th>Slope (days/min)</th>
<th>TTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0†</td>
<td>0.373 ± 0.010</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>0.118 ± 0.005</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>8</td>
<td>0.061 ± 0.001</td>
<td>4.6 ± 0.1</td>
</tr>
<tr>
<td>16</td>
<td>0.076 ± 0.004</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td>24</td>
<td>0.078 ± 0.006</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td>48</td>
<td>0.095 ± 0.004</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>72</td>
<td>0.134 ± 0.004</td>
<td>2.8 ± 0.1</td>
</tr>
<tr>
<td>120</td>
<td>0.199 ± 0.008</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>168</td>
<td>0.330 ± 0.016</td>
<td>1.1 ± 0.1</td>
</tr>
</tbody>
</table>

† Single heat treatment.

Discussion

The kinetics of thermotolerance in a human melanoma xenograft was studied by using tumor volumetric tripling time as end point. The ratio of the slopes of the dose-response curves for preheated and single-heated tumors (Chart 3) was used as a quantitative measure of the developed thermotolerance (Table 1).

The kinetics of thermotolerance is presented in Chart 4 where TTR is shown as a function of fractionation interval. Thermotolerance developed rapidly to a maximum of 4.9 ± 0.3 at 16 hr, followed by a slow decay to 1.1 ± 0.1 at 168 hr. It should be noticed that the TTR values determined for the longer fractionation intervals, especially for 168 hr, to some extent may have been influenced by the analysis procedure. (a) The regrowth curve in Chart 1a for tumors exposed to a single heat treatment of 30 min indicates that regrowth was initiated during the first week (168 hr) after treatment, i.e., before the second heat exposure for the longer fractionation intervals. This regrowth probably reduced the effect of the second treatment and hence shortened the tumor volumetric tripling time for the longer relative to that for the shorter fractionation intervals. (b) Tumor volumetric tripling time was defined as the time from the first treatment to the time the tumor volumes had reached 3 times the initial volumes. This definition overestimates the effect of the second treatment and hence contributed to enhance the tumor volumetric tripling time for the longer relative to that for the shorter fractionation intervals. Nevertheless, the kinetics of thermotolerance in the xenograft was qualitatively similar to that observed for cells in culture (8, 9, 15, 18, 19, 36, 37), murine normal tissues (16, 21, 23, 28–30, 44, 50), and experimental rodent tumors (25, 34, 39, 51, 52). However, as discussed below, there are some quantitative differences.

Several authors have suggested the development of thermotolerance in experimental, solid tumors (5, 25, 34, 39, 41, 51, 52). However, in only 3 of these studies, TTR was defined as
The ratio of the slopes of dose-response curves for preheated and single-heated tumors, i.e., the studies of Kamura et al. (25) and Nielsen and Overgaard (39) on the murine C3H mammary carcinoma and the study of Wheldon et al. (52) on the rat sarcoma SSB1a. Thermotolerance in these tumors is compared with that in the melanoma xenograft in Chart 5. Kamura et al. (25) showed that TTR for the C3H mammary carcinoma reached a maximum of $5.2 \pm 1.1$ at 16 hr and that TTR decayed to $1.0 \pm 0.1$ at 120 hr. The priming heat dose in this study was $43.5^\circ$ for 30 min. The kinetics of thermotolerance in a given biological system appears to depend on the effect of the priming heat dose. The time interval necessary to obtain maximum thermotolerance and
the time interval necessary for complete decay of thermotolerance increases with increasing priming heat dose (30, 38, 39). Consequently, the results from the study of Kamura et al. (25) cannot be compared directly with those from the present experiments where the priming heat dose was 42.5°C for 30 min. Nielsen and Overgaard (39), using the same tumor and the same experimental procedure as Kamura et al. (25), showed that maximum TTR for a priming heat dose of 43.5°C for 15 min was 3.7 ± 0.4 and appeared 8 hr after treatment (Chart 5). Since a decrease in temperature of 1°C often is assumed equivalent to an increase in heating time by a factor of 2 (7), a priming heat dose of 43.5°C for 15 min is approximately equivalent to that used in the present study. Bearing in mind the relationship between the kinetics of thermotolerance and the priming heat dose (30, 38, 39), a comparison of the present data with those of Kamura et al. (25) and Nielsen and Overgaard (39) shows that (a) maximum TTR is higher for the melanoma xenograft than for the C3H mammary carcinoma and (b) the time necessary for complete decay of thermotolerance is longer for the melanoma xenograft than for the C3H mammary carcinoma. TTR for the rat sarcoma SSB1a was determined only at 24 and 48 hr after a priming heat dose of 43.5°C for 30 min (Chart 5). The data indicate that the thermotolerance induced in this sarcoma by a given treatment is higher than that induced in the C3H mammary carcinoma but lower than that induced in the melanoma xenograft.

The present results may have some implications for the choice of fractionation regimens in clinical treatment of cancer with hyperthermia. The optimum fractionation interval will probably depend on the magnitude and the kinetics of thermotolerance induced in the tumor and in the surrounding normal tissue. If the thermotolerance induced in the normal tissue is larger than that in the tumor, a therapeutic gain is likely to result for treatment regimens with fractionation intervals longer than the time necessary to reach maximum thermotolerance in the normal tissue. This assumption is based on the observations reported for cells in culture that (a) the time necessary to reach thermotolerance maximum increases with increasing thermotolerance maximum and (b) the rate of decay of thermotolerance is independent of thermotolerance maximum (18, 32, 33, 38). However, if the thermotolerance induced in the tumor is larger than that induced in the normal tissue, the normal tissue may suffer more heat damage than the tumor. The magnitude and the kinetics of thermotolerance induced by equal treatments appear to vary considerably among different normal tissues (16, 21, 23, 28–30, 44, 50) and different tumors (25, 34, 39, 51, 52) (Chart 5). Heterogeneous tumor heating, due to vascular cooling (2, 20, 22, 35) or inadequate heating techniques (14, 40, 42, 48), as well as the presence of tumor cell subpopulations with different heat sensitivities (Refs. 31 and 49; Footnote 4) may result in a considerable variation in the thermotolerance within individual tumors. These complications indicate that it may be extremely difficult to predict the thermotolerance in a given tumor and in the limiting normal tissue clinically and hence to prescribe fractionation regimens based on differences in thermotolerance between these tissues. The problems related to the development of thermotolerance may be considerably reduced by using treatment regimens with fractionation intervals sufficiently long to ensure complete decay of thermotolerance. A therapeutic gain is then to be expected on the basis of differences between the tumor and the normal tissue in temperature and physiology. The following question then arises: how long should the interval between 2 heat fractions be? The thermotolerance induced in the melanoma xenograft by a heat dose of 42.5°C for 30 min decayed completely within 7 days (168 hr) after treatment. If temperatures above 42.5°C are maintained for 30 min or longer, complete decay of thermotolerance will probably require more than 7 days. Regrowth was initiated in the melanoma xenograft before the thermotolerance had decayed completely, but regrowth between 2 heat treatments will probably not occur in clinical practice, since hyperthermia is used commonly in combinations with radiation or chemotherapy. Consequently, the present results indicate that treatment protocols probably should not prescribe more than one hyperthermic treatment per week.

ACKNOWLEDGMENTS

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