Effect of Hyperglycemia on the Thermal Response of Murine Normal and Tumor Tissues

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ABSTRACT

The effect of an i.p. injection of glucose on the thermal response of murine tissues was studied. Animal tumors were early generation isografts of a spontaneous fibrosarcoma, FSa-II. Tumors were transplanted into the foot pad, and hyperthermia was given by immersing the foot into a constant-temperature water bath. The tumor and normal tissue responses were studied by assays of the time required for half the tumors to reach 1000 cu mm from treatment day and of the treatment time required for one-half of the animals to develop a loss of one toe or greater reaction. The glucose administration enhanced tumor response more substantially than normal tissue response. The enhancement was greater for a large tumor than for a small tumor and also greater at 42.0°C than at 45.5°C. Presumably, the hyperglycemia induced acidosis which eventually enhanced thermal response. Present results suggested that the hyperglycemia is a potential method to specifically enhance tumor response at elevated temperatures.

INTRODUCTION

Biological effect of hyperthermia has been extensively studied in mammalian cells as a potential modality for human cancer treatment (1, 14). Hyperthermia at 41–46°C can kill mammalian cells and sensitize the response to radiation or chemotherapy. Recent studies have revealed that a decrease in environmental pH is associated with increasing thermal sensitivity (3, 5, 12). Animal tumors contain a fraction of hypoxic cells which undergo anaerobic glycolysis with a resultant accumulation of lactic acid (8). In this situation, tumor tissues may be more acidic and, as a result, more sensitive to heat than the normal tissues. Direct measurements of tumor tissue pH confirmed that the tumor tissue pH was lower than the normal tissue or blood pH (8–10). The pH enhancement is predominant at decreased pH (3). This suggests artificial acidosis may further enhance the response of tumors more substantially than that of normal tissues. We have investigated the effect of artificial acidosis on the thermal response in animal tumor and normal tissues. In this paper, hyperglycemia was used to induce metabolic acidosis.

MATERIALS AND METHODS

Animals were 10- to 12-week-old C3H/Sed mice derived from and kept in our defined flora mouse colony (13). Animals were provided Wayne Lab Blox and sterilized, vitamin K-fortified water ad libitum. Tumors were eighth generation isografts of a fibrosarcoma which arose spontaneously in a female C3H/Sed mouse. The tumor is designated FSa-II. Single-cell suspensions were prepared by trypsinization, and 5 µl of the suspension containing ≈ 10⁶ viable tumor cells were injected into the right foot pad. The method of preparing the suspension is fully described elsewhere (15, 16).

Hyperthermia was given in a water bath where a desired temperature ± 0.1°C was maintained by a constant temperature circulator (16). Animals were held in specifically designed holders without anesthesia, and animal feet were immersed in a water bath. Tumor temperature was equilibrated within 90 sec and was no less than 0.1°C below the water bath temperature. The response of tumors and normal tissues was studied by the TG time and RD₉₀ assays described in detail elsewhere (16, 17). Animal tumors were treated when they reached an average diameter of 4 or 8 mm. Following the treatment, 3 diameters of each tumor, a, b, and c, were measured at least 3 times a week until they exceeded an average diameter of 13 mm, and the volume was calculated by πabc/6. The tumor growth curves were constructed for each tumor, and the TG time was obtained graphically. The median TG time was then calculated by logit analysis (16).

The reaction of non-tumor-bearing foot was examined according to our numerical score system (17). Following the hyperthermia, the foot reaction was scored twice a week for 5 weeks. Based on the frequency of animals which developed a score of 4.0 (loss of one toe) or greater reaction, the RD₉₀ was calculated.

The glucose (50% dextrose) was given i.p. 60 min before hyperthermia.

RESULTS

The 4- or 8-mm FSa-II tumors were treated at 42.0°C or 45.5°C, and the TG times were studied. At 45.5°C, an exponential relationship with an initial shoulder was found between the TG time and treatment time (Chart 1). The dose-response curve for tumors pretreated with glucose was steeper than that for tumors receiving no pretreatment, and the shoulder was reduced, indicating that the glucose enhanced the response of FSa-II tumors to hyperthermia. The enhancement appeared to depend on the glucose dose. The glucose ER was calculated as a ratio of the treatment time which results in a TG time of 20 days for the control to that for glucose. As shown in Table 1, the ER was obviously greater for 8-mm tumors than for 4-mm tumors. It is also notable that the slope of the dose-response curve for 8-mm tumors was steeper than that of the dose-response curve for 4-mm tumors. This confirmed our previous observation that a large tumor was more sensitive to heat than was a small tumor (16).

Tumors treated at 42.0°C without glucose pretreatment exhibited a biphasic dose-response curve, indicating a development of thermal resistance during the treatment (Chart 2). The hyperglycemia did not appear to inhibit the development of...
thermal resistance in the 4-mm tumor. The glucose enhancement was appreciably greater for the 8-mm tumor with inhibition of thermal resistance. In addition, the ER for the 8-mm tumor was greater at 42.0 ° than at 45.5 ° (Table 1).

The RD(90) values (95% confidence limit) for animals pretreated with 0, 5, and 10 mg glucose per g were 36 (range, 34 to 39), 31 (range, 29 to 33), and 29 (range, 26 to 33) min at 45.5 °, respectively. The therapeutic gain at this temperature was greatest for the 8-mm tumor pretreated with 10 mg glucose per g. The RD(90) values at 42.0 ° with or without 10 mg glucose per g were 480 (range, 410 to 560) and 680 (range, 630 to 720) min, respectively, resulting in a therapeutic gain of 1.76 for the 8-mm tumor (Table 1).

**DISCUSSION**

The acidosis can be induced by a variety of methods including an excess CO₂ in respiratory air or by hyperglycemia. The latter results in metabolic acidosis, while the former leads to respiratory acidosis. Presumably, the hyperglycemia stimulates release and production of insulin which eventually facilitates all pathways of glucose metabolism. In hypoxic cells in a tumor, the anaerobic glycolysis may be enhanced with a resultant accumulation of lactic acid. Our preliminary measurement of blood pH demonstrated that the blood pH was reduced to 6.8 from a normal value of 7.3 within 1 hr following an i.p. injection of 10 mg glucose per g and returned to original value in approximately 6 hr. Eden et al. (2) investigated the pH response to glucose injection of rat normal and tumor tissues. Although the pH change in normal tissue was identical to our observation in the blood, the tumor pH decreased more substantially and remained low for a more prolonged period than did the normal tissue pH. They found the minimum tumor pH of =6.5 at 6 hr following the glucose injection. Comparable results have been observed by other investigators (7, 10). Another action of hyperglycemia is to increase osmotic pressure in the extracel-

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**Table 1**

<table>
<thead>
<tr>
<th>Glucose dose (mg/g)</th>
<th>4 mm Tumor</th>
<th>8 mm Tumor</th>
<th>Foot</th>
<th>Therapeutic gain for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T(90) a (min)</td>
<td>ER</td>
<td>T(90) b (min)</td>
<td>ER</td>
</tr>
<tr>
<td>at 45.5 °</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>26.7 ± 3.6 b</td>
<td>1.0</td>
<td>25.1 ± 2.8</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>25.3 ± 3.2</td>
<td>1.06</td>
<td>21.1 ± 2.7</td>
<td>1.19</td>
</tr>
<tr>
<td>10</td>
<td>20.1 ± 3.2</td>
<td>1.33</td>
<td>16.1 ± 1.8</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>T(90) d (min)</td>
<td>ER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 42.0 °</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>225</td>
<td>1.0</td>
<td>680 (650–720)</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>165</td>
<td>1.36</td>
<td>520 (470–560)</td>
<td>1.32</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>2.5</td>
<td>480 (410–560)</td>
<td>1.42</td>
</tr>
</tbody>
</table>

a T(90) or T(10), treatment time at elevated temperature to prolong TG time to 20 or 10 days, respectively.
b Mean ± 95% confidence limits.
c Numbers in parentheses, range.
d Dose-response curves fitted by eyes.
lular fluid, resulting in intracellular dehydration which may cause relative increase of lactic acid concentration. Accordingly, hyperglycemia could reduce tissue pH by 2 different mechanisms.

Our previous experiment demonstrated that a large tumor was more sensitive to heat than was a small tumor, suggesting that the tumor tissue pH decreased as tumors grew. The estimated pH values of the 4- and 8-mm tumors were ≈7.4 and ≈7.0, respectively (16). In this situation, the hyperglycemia may enhance the pH effect more substantially in a large 8-mm tumor than in a small 4-mm tumor with an increased therapeutic gain for the 8-mm tumor as observed in the present study.

It has been demonstrated in cultured mammalian cells that the pH enhancement is predominant with decreasing pH and is more substantial below 43.0° than above 43.0° (3). Present results confirmed that these features of the pH effect found in cultured cells were comparable to those observed in animal tumors. It is notable, however, that a glucose dose of 10 mg/g is safe but corresponds to approximately three-fourths of the 50% lethal dose of glucose. Accordingly, it appears to be essential to establish a treatment method to enhance the thermal response at reduced glucose dose. Besides, it is still unknown how the hyperglycemia modifies thermotolerance in vivo, although it has been demonstrated that the decreased medium pH reduced the magnitude of thermotolerance (4, 6, 11).

REFERENCES

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