Enhancement by Corynebacterium parvum of the Normal and Tumor Tissue Response to Hyperthermia

Muneyasu Urano, Marie Overgaard, Herman Suit, Paul Dunn, and Robert Sedlacek

Edwin L. Steele Laboratory of Radiation Biology, Department of Radiation Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114

ABSTRACT

The effect of Corynebacterium parvum treatment on the response of tumor and normal tissue to hyperthermia (43.5°) was studied. Animals were C3Hf/Sed mice from our defined flora mouse colony. The time at hyperthermia that achieved control of one-half of methylcholanthrene-induced fibrosarcomas and the foot reaction were examined after treatment. C. parvum, if given 3 to 32 days before hyperthermia, enhanced the reaction to local hyperthermia of normal tissue. No enhancement was observed if C. parvum was given after hyperthermia. This enhancement was more dramatic for tumor response resulting in a therapeutic gain factor of \(-2.3\) (3.7/1.6). Comparative studies on combined Corynebacterium and radiation failed to demonstrate the enhancement to normal tissue.

INTRODUCTION

Administration of C. parvum to intact laboratory animals has been extensively studied; it stimulates the reticuloendothelial system (1), augments macrophage function (6, 13, 14), and for some tumor systems retards tumor growth or occasionally causes complete regression of established tumors (2, 7, 9, 11). Clinical trials or laboratory experimental studies with C. parvum usually feature the combination of C. parvum with other treatment modalities (3, 4, 10, 12). The use of the bacterium in combination with radiation therapy, for example, is based on the expectation that C. parvum retards tumor growth or occasionally causes complete regression of established tumors (2, 7, 9, 11). Clinical trials or laboratory experimental studies with C. parvum usually feature the combination of C. parvum with other treatment modalities (3, 4, 10, 12). The use of the bacterium in combination with radiation therapy, for example, is based on the expectation that C. parvum potentiates the host immune reaction against the established tumor may result in reduction of the radiation dose required to achieve tumor control and hence in a lesser degree of normal-tissue damage. In a recent experiment we found that hyperthermia following i.v. injection of C. parvum can eradicate mouse fibrosarcoma more effectively than does hyperthermia alone. This report describes the response of normal and tumor tissue to hyperthermia in normal or C. parvum-treated hosts. Results from comparable studies with C. parvum and radiation are also presented.

MATERIALS AND METHODS

C3Hf/Sed mice, 8 to 12 weeks old, from our defined flora mouse colony were used in the assays (11). Tumors were fifth-generation isotransplants of fibrosarcoma that had been induced by a single injection of methylcholanthrene (11). Transplantation was achieved by injecting 2 to 5 \times 10^5 viable tumor cells (trypan blue exclusion test) into the right foot. For hyperthermia the foot was immersed in an constant-temperature water bath, 43.5 ± 0.1°. The temperature of the foot was not less than 43.3°. Local irradiation was performed with the use of parallel opposed 137Cs beams (3 cm in diameter) (8). The mice were unanesthetized for heat or radiation treatments.

C. parvum was provided by Burroughs Wellcome and Co. (Research Triangle Park, N. C.) as formalin-killed organism suspended in thiomersalate. C. parvum was administered as single i.v. doses of 350 \(\mu\)g (in 0.4 ml). After treatment animal feet were examined at least 4 times/week for 35 days, and the foot reaction was scored according to this protocol: 0, normal foot; 0.5, 50% chance of damage; 1.0, red foot, epilation; 1.5, slight edema; 2.0, severe edema, complete epilation; 2.5, fusion of toes; 3.0, partial wet desquamation; 3.5, wet desquamation of most of foot; 4.0, loss of 1 toe; 4.5, loss of more than 2 toes; 5.0, loss of foot; 6.0, loss of leg up to the ankle. This scoring system includes the response of not only the skin but also the bones. Scores of 0 to 3.5 represented skin response and the damage was reversible, while scores of 4.0 to 6.0 represented bone necrosis and the damage was irreversible. The first series of experiments in which foot reaction was scored for 42 days revealed no increase of the reaction after 30th posttreatment day. Therefore, in later experiments foot reactions were scored for 35 days.

Responses of normal feet are described with the use of 2 end points: (a) average peak reaction, \(i.e.,\) the average of maximum foot reaction of each mouse observed between the fifth and 35th posttreatment day; and (b) \(RD_{0.5}\) \(4+\) (4+ reaction) values. Tumor responses are given as \(TCD_{50}\) values. Tumors were 7 mm in diameter at treatment. Animals were randomly assigned into experimental groups. This procedure kept observers blind for groups at the time of scoring, \(i.e.,\) observers were unable to know which animals had received C. parvum and which had not. Thirty to 35 mice were used for an assay. Computational methods for \(RD_{50}\) and \(TCD_{50}\) values have been described (10).

\(5\) The abbreviations used are: \(RD_{50}\), time at hyperthermia that elicits a 1 reaction of normal tissue in one-half of the heated feet; \(TCD_{50}\), time at hyperthermia that achieves control of one-half of the heated tumors; ER, enhancement ratio [ratio of treatment time to induce a given reaction (TCD or RD) in animals receiving no C. parvum to treatment time in C. parvum-treated mice].

\(6\) The abbreviations used are: \(RD_{50}\), time at hyperthermia that elicits a 1 reaction of normal tissue in one-half of the heated feet; \(TCD_{50}\), time at hyperthermia that achieves control of one-half of the heated tumors; ER, enhancement ratio [ratio of treatment time to induce a given reaction (TCD or RD) in animals receiving no C. parvum to treatment time in C. parvum-treated mice].
RESULTS

Chart 1 presents the average peak foot reaction as a function of time at 43.5° in normal and C. parvum-treated mice. Reactions of feet of mice that received 350 µg C. parvum i.v. 3 days before hyperthermia were clearly more severe than those in animals who received hyperthermia alone. The ER's were 1.48, 1.53, or 1.55 at reaction scores of 1+, 3+, or 5+, respectively, i.e., ER's were independent of the magnitude of foot damage. Similar effects were observed when C. parvum was given 8 or 32 days before hyperthermia (Chart 1B). The slopes of dose-response relations of animals pretreated with C. parvum 3 to 32 days before hyperthermia were all significantly steeper than that of animals who received hyperthermia only ($p < 0.01$). The foot reactions of animals given C. parvum immediately or 3 days after the heat were not enhanced (Chart 1C).

RD$_{50}$'s (4+ score) are tabulated in Table 1 for 2 experiments. As for peak reactions the RD$_{50}$ (4+ score) was significantly shorter in mice given C. parvum 3 days before hyperthermia. The ER for the pooled data was 1.45. RD$_{50}$ (4+ score) values for animals who received C. parvum 8 or 32 days before hyperthermia were 104 (ER = 1.28) or 97 (ER = 1.37) min, respectively; for animals who received C. parvum immediately or 3 days after hyperthermia, the RD$_{50}$ values were 137 (ER = 0.97) or 135 (ER = 0.99) min, respectively.

TCD$_{50}$ values for the 7-mm tumor were 92.6 and 25.0 min for control and C. parvum-treated mice (3 days before hyperthermia), respectively. ER for tumor response was 3.7 (92.6/25.0). In this experiment foot reactions were scored in animals in which tumors were controlled by hyperthermia or combined treatments. RD$_{50}$ (4+ score) values were greater in animals who carried tumors than they were in nontumorous mice. However, the ER of 1.61 was not different from the ER of 1.45 for the assay with normal feet (Table 2). In this system, the therapeutic gain factor is 2.30, i.e., despite an enhancement of normal tissue reaction, there was a net improvement in therapeutic results.

Comparable studies were made on the foot reaction of animals treated with C. parvum and irradiation. C. parvum was given 3 or 8 days before irradiation, and average peak reactions were plotted as a function of radiation dose. The dose-response curve of these animals was not significantly shifted to the left of that for animals treated with radiation alone. Foot reactions in mice who received radiation 7 or 14 days before C. parvum were almost exactly the same as those in animals who received radiation alone (Chart 2).

DISCUSSION

The results demonstrated that C. parvum enhanced the response of normal foot tissue to hyperthermia but not to radiation. Nonetheless, there was a clear therapeutic gain in the C. parvum-treated mice, where there was a greater increase in response of this moderately strongly immuno-

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Hyperthermia alone</th>
<th>C. parvum + hyperthermia</th>
<th>ER</th>
<th>$p$ (t test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay 1</td>
<td>125 (113-138)</td>
<td>82 (67-101)</td>
<td>1.62</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Assay 2</td>
<td>140 (131-152)</td>
<td>96 (90-103)</td>
<td>1.39</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pooled</td>
<td>133 (126-141)</td>
<td>92 (83-103)</td>
<td>1.45</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

$^a$ C. parvum was given 3 days before hyperthermia.

$^b$ Numbers in parentheses, 95% confidence limit.
Table 2

Effect of C. parvum and hyperthermia in treatment of mouse fibrosarcoma growing in footpad

<table>
<thead>
<tr>
<th>End point</th>
<th>Hyperthermia alone</th>
<th>C. parvum + hyperthermia</th>
<th>Therapeutic gain factor</th>
<th>p (t test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCD50 (min)</td>
<td>92.6 (76.6–112.0)*</td>
<td>25.0 (14.4–43.0)</td>
<td>3.70</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>114 (109–120)</td>
<td>71 (66–77)</td>
<td>1.61</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Time interval between treatment was 3 days.
* Numbers in parentheses, 95% confidence limit.

lethal heat damage might be sensitive target cells for C. parvum-activated macrophages. Although the mechanism for the C. parvum-mediated sensitization of normal tissue to hyperthermia is obscure, the data from these experiments constitute a clear caution against combining C. parvum with standard heat treatments, i.e., if C. parvum is to be combined with hyperthermia, caution should be taken when deciding the thermal treatment schedule. If the ER for tumor response were less than that for normal tissue response, the therapeutic gain factor would be less than 1.00 (i.e., not an acceptable treatment).

REFERENCES

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