IV. 2 Tumor Growth Delay Caused by Proton Therapy in Combination with the Vascular Disrupting Agent AVE8062

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Vascular disrupting agents (VDAs) are designed to cause a rapid and selective blood flow interruption in tumors leading to extensive tumor necrosis as a result of oxygen and nutrient deprivation. In spite of the fact that VDAs give rise to a catastrophic shutdown in the vascular function of the tumor, the tumor cells at the border between malignant and normal tissue survive treatment with VDAs. It is believed that the tumor cells at the tumor periphery are supported by oxygen and nutrients from the surrounding normal vessels and repopulate the tumor after the VDA treatment alone1). Thus, VDAs may fully provide their therapeutic potential when used in combination with radiotherapy or chemotherapy. In the present study, we aim to evaluate the therapeutic efficacy of proton therapy combined with the vascular disrupting agent AVE8062, which is undergoing clinical assessment, by using the tumor growth delay (TGD) assay in a solid murine tumor.

The proton therapy experiment was performed at the proton therapy facilities2) of Cyclotron and Radioisotope Center (CYRIC), Tohoku University. NFSa fibrosarcoma cells (5×10⁶/50 μL) were transplanted into both hind legs of C3H/HeSlc male mice aged around 12 weeks old. Figure 1 illustrates a beam delivery technique in the present experiment. When each tumor diameter reached about 8 mm, single-dose irradiation was given only to the tumor of the right hind leg. In order to immobilize the mouse during the irradiation, pentobarbital anaesthesia (50 mg/kg) was administered intraperitonealy to each mouse. The tumor was irradiated in the maximum depth dose region, the so-called SOBP
(spread-out Bragg peak) provided with an energy modulation filter, and received a single dose of 15 or 30 Gy at 5 Gy/min. The SOBP width was 10 mm. The normal tissue surrounding the tumor was shielded from the irradiation with patient’s collimator and bolus. The tumor of the left hind leg was not irradiated. In addition, AVE8062 was administered intraperitonealy to a part of the mice receiving 15 Gy at a dose of 40 mg/kg 2 hours after irradiation. As a result, we classified the tumors of the right and left hind legs into proton therapy (15 or 30 Gy), AVE8062 treatment (40 mg/kg), combination treatment (15 Gy + 40 mg/kg) and control groups, as shown in Fig. 2. All experimental protocols in this work were reviewed by the Committee on the Ethics of Animal Experiments at Tohoku University, and were performed in accordance with its guidelines.

Figure 3 shows the time-course of tumor volume for each treatment group. The tumor volumes were measured daily after the treatment according to the formula \( \pi abc/6 \), where \( a, b \) and \( c \) are three orthogonal diameters of the tumor. In this work, TGD was defined as the difference in tumor growth time in days required for each tumor volume to reach four times the initial volume between the control and the treatment groups\(^3\). Results of TGD observed for the treated tumors are listed in Table 1. Although TDG for the proton therapy at a single dose of 15 Gy was about 2 days and approximately equal to that for AVE8062 treatment alone, the combination treatment inhibited the tumor growth more strongly and enhanced TGD at levels nearly comparable to that for the proton therapy at a single dose of 30 Gy.

In conclusion, the significant enhancement of tumor growth delay in the proton therapy combined with post-radiotherapy administration of AVE8062 was observed in the present work. A possible explanation for the present result is that the combination treatment may efficiently kill radiation-resistant hypoxic cells in the tumor as well as the tumor cells at the tumor periphery which survive monotherapy with VDAs.

This work was supported by Grants-in-Aid for Scientific Research (B) Nos. 17300169 (A. Terakawa) and 20300174 (A. Terakawa), and by Exploratory Research No. 19650128 (A. Terakawa) of the Ministry of Education, Culture, Science, Sports and Technology.

References

Table 1. Tumor growth delay (TGD) for the treated tumors.

<table>
<thead>
<tr>
<th></th>
<th>Proton therapy at 15Gy (n = 4)</th>
<th>AVE8062 administration (40 mg/kg) (n = 5)</th>
<th>40 Proton therapy at 30 Gy (n = 4)</th>
<th>AVE8062 administration (40mg/kg) 2 h after proton therapy at 15 Gy (n = 5)</th>
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<tbody>
<tr>
<td>TGD (day)</td>
<td>2.1±1.1</td>
<td>2.8±0.7</td>
<td>9.2±1.1</td>
<td>7.4±0.7</td>
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Figure 1. Dose delivery to the tumor in proton therapy.
Figure 2. Groups of the treated tumors in the present study.

Figure 3. Time course of the relative tumor volumes. Each single-treatment was done at day 0. Arrows indicate the TDGs for the treated tumors.