IV. 3. Radiotherapeutic Effects on the Tumor Uptake of $^{11}$C-L-Methionine II: Monitoring of Tumor Recurrence


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Introduction

There is an emerging need for oncologic patient evaluation method which can monitor disease and can predict the response before the ultimate results of treatment becomes clear.\(^1\) Positron emission tomography using metabolic tracers $^{18}$F-fluorodeoxyglucose or $^{11}$C-L-methionine has made it possible to study the biological property of individual tumor which include benign or malignant, grade of malignancy and the prognosis.\(^2-7\) Biologically useful informations rather than anatomic have been expected in the application of PET to cancer treatment evaluation.

Previously, we have reported that tumor uptake of L-[methy$^{11}$C]methionine (MET) responded to radiotherapy much more sensitively than does the tumor volume reduction and the extension of necrosis.\(^8\) Its clinical application using PET is a candidate for new treatment evaluation method. In this report, we further studied about the dose-response of MET tumor uptake to irradiation in order to examine the ability to monitor the tumor recurrence.

Materials and Methods

Tumors and irradiation

A 0.1 ml of suspension containing $7 \times 10^6$ cells of ascitic hepatoma AH109A was subcutaneously inoculated in a thigh of young male Donryu rats weighing from 180 to 220 g. Irradiation was performed when a transplanted tumor grew to 1.5 to 2.0 cm in diameter. A rat was anesthetized and fixed to place the tumor-bearing thigh in the field of irradiation
and the tumor was exposed to 5 to 20 Gy of single dose cobalt-60\(^{(60\text{Co})}\) irradiation as described previously.\(^8\) 

**Tumor growth study**

Solid tumor on the thigh was measured with a vernier caliper every day during experiment. The product of the three principal diameters of the tumor was designated as tumor volume.\(^9\) Two groups of eight rats each were used for tumor recurrence study with or without 10 Gy irradiation.

**MET uptake study**

For dose-response study, three groups each including 8 or 7 rats were administered MET, 48 hours after the irradiation of 5, 10 or 20 Gy, and one group without irradiation was used as a control. For tumor recurrence study, four groups of 6 to 8 rats were administered MET, 2, 6, 8 and 10 days after 10 Gy irradiation, and a group without irradiation was used as a control.

MET was synthesized as described previously.\(^5\) The radiochemical purity was over 99%. After fasting for 8 hours, about 100 μCi of MET was i.v. injected through the tail vein of a rat that was killed 30 min later. Tissue samples were excised and weighed, and the radioactivities were counted by an auto-gamma counter. The tissue radioactivity was expressed as differential uptake ratio (DUR = tissue counts per gram/ injection dose counts per body weight).

**Results**

Dose-response of irradiation on the tumor uptake of MET was shown in Table 1. Tumor uptake of MET in the groups of 5, 10 and 20 Gy were significantly lower than the control, also that of 10 and 20 Gy were significantly lower than the 5 Gy, but no significant difference between the 10 and 20 Gy groups. The femur with bone marrow from the irradiated thigh showed the same pattern of MET uptake changes. The blood level and the uptake by the muscles remained low and constant.

Fig. 1 showed the correlation of the tumor volume and MET uptake along the time after irradiation. The non-irradiated group was plotted at time 0. After a 10 Gy of irradiation, tumors continued to enlarge until Day 2 (174±37% of the control, p<0.001), then began to shrink. On Day 6 and 7, tumor volume was the smallest (84±27%, 84 ±33%). The tumor started to regrow on Day 8 and showed significant regrowth on and after Day 9 (155±55%, p<0.01 compared to Day 7). The MET uptake by the tumor dropped sharply on Day 2 (30±14%, p<0.001) and was almost constant till Day 6.
(32±22%), then showed significant increase on Day 8 (61±19%, p<0.05, compared to Day 6), and reached the level of non-irradiated group on Day 10 (98±10%).

Discussion

There are number of reports that some tumor mass remain after successful treatment of lymphoma and testicular carcinoma.\textsuperscript{10-13} Also regression rate of tumor volume was not correlated with the tumor control incidence.\textsuperscript{14} Tumor volume measured by radiological techniques is the most popular indicator for cancer treatment but not the best indicator for viability in some tumors. Tumor containing non-active tissue; fibrosis, necrosis and injured cell on the way to death will produce the diagnostic problem that the residual mass may not be residual diseases\textsuperscript{15}. Monitoring technique which provide information about the nature of the mass lesion rather than the size will be important to avoid unnecessary treatment for the patients and to measure true efficacy of a particular treatment.

Monitoring the treatment response to radiotherapy has been studied with FDG both in experimentally\textsuperscript{16} and clinically\textsuperscript{17-19}. Tumor uptake of FDG responded to the treatment very well and showed high activity in case of recurrence, but its correlation to the volume change were not yet studied. MET tumor uptake showed the discrepancy between the volume changes of tumor after radiotherapy in this study. Although the tumor volume continued to increase until 2 days after irradiation, MET tumor uptake dropped to 30% of the non-irradiated rapidly. When the tumor started to regrow late after irradiation, significant increases of MET tumor uptake was observed earlier than the increase of the tumor volume. Our results clearly showed the superiority of metabolic tracer study over the tumor volume study for the monitoring of tumor radiotherapy.

In the dose-response study, MET tumor uptake showed linear decrease with significant difference from the control to the 10 Gy, the 20 Gy was lower than the 10 Gy but not significant. This AH109A tumor showed regrowth after 10 Gy of irradiation but did not after 20 Gy of irradiation. It arise a question that can MET tumor uptake study differentiate if the tumor was sterilized completely or not. This study was performed 2 days after the irradiation, and we can estimate the residual viable cell at this point using the tumor growth curve of 10 Gy irradiated tumor(Fig 2). If the rate of tumor regrowth is the same as the control, we can extrapolate the regrowth fraction of tumor from Day 8 to Day 2. The regrowth fraction of tumor on Day 2 was about 1 % of the control and the gross tumor volume was 174%. It means only 0.57% (1/174) of cells in a palpable tumor is actively growing on Day 2 after 10 Gy, whereas 0% after 20 Gy. And we measured gross tumor radioactivity calibrated by the tumor weight. When we reviewed accuracy and variation of this experiment, discrimination of these two is impossible.
Tumor regrowth after irradiation has been well studied and accelerated regeneration has been observed in various tumors where the regrowth fraction is lower than the simple extrapolation in early after irradiation due to the sigmoid curve.\textsuperscript{20} And 1\% in this curve seems to be the highest estimation. Dose-response from the control to 10 Gy demonstrated that MET tumor uptake clearly represented the gross changes of tumor viability. Our study suggested that MET tumor uptake is more sensitive indicator for radiotherapy and for tumor recurrence than the tumor volume measurement. But, in order to differentiate if the tumor was sterilized completely or not in early after irradiation, the measurement system of MET tumor uptake must be more accurate than this study.

References

14) Suit H., Lindberg R., Fletcher G. H., Radiology 84 (1965) 1100.
Table 1. Dose-response of 48hr after $^{60}$Co Irradiation on $^{11}$C-L-Methionine uptake by AH109A tumor 30 min after i.v.

<table>
<thead>
<tr>
<th>DUR of tissue (Mean ± SD) (n)</th>
<th>Control</th>
<th>5Gy</th>
<th>10Gy</th>
<th>20Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>3.187±0.615(7)</td>
<td>2.343±0.397* (8)</td>
<td>1.837±0.487† (7)</td>
<td>1.658±0.238NS (7)</td>
</tr>
<tr>
<td>Bone+Marrow</td>
<td>1.308±0.063(7)</td>
<td>0.878±0.080** (8)</td>
<td>0.740±0.107† (7)</td>
<td>0.688±0.080NS (8)</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.899±0.063(7)</td>
<td>0.837±0.071(8)</td>
<td>0.828±0.061(7)</td>
<td>0.838±0.056(7)</td>
</tr>
<tr>
<td>Blood</td>
<td>0.344±0.034(7)</td>
<td>0.355±0.021(8)</td>
<td>0.359±0.040(7)</td>
<td>0.367±0.024(8)</td>
</tr>
</tbody>
</table>

Differential Uptake Ratio (DUR) = \( \frac{\text{Tissue Activity/ Body Weight (g)}}{\text{Tissue Weight(g)/Injection Activity}} \)

Student's t test

* p<0.01 compared to the control
** p<0.001
† p<0.05 compared to 5Gy

NS not significant compared to 10Gy
Fig. 1. Tumor volume curve correlated to MET tumor uptake curve after 10 Gy of irradiation. Non-irradiated control values were plotted on Day 0 as 100 %. Normal scale.

Fig. 2. Tumor volume curve was analyzed on the logarithm-normal plot. Day 0 is day of 10 Gy irradiation and data obtained before irradiation was plotted as 100 %. Tumor regrowth curve was extrapolated back to day 2 (broken line) when dose-response study of MET tumor uptake was done.