

Radiolabeled Thymidine: A Sensitive Tracer for Early Tumor Response and Recurrence After Irradiation

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This study evaluated the sensitivity of a radiolabeled thymidine tracer for assessment of early tumor response and recurrence after irradiation. **Methods:** SW707 human colon carcinoma implanted into nude mice was irradiated with 6 or 20 Gy. Tumor volume was determined for an interval of 14 d. At 4, 8 and 24 h and at 2, 3, 7, 10 and 14 d after irradiation, [¹⁴C]thymidine uptake into the tumor was determined with a liquid scintillation counter and the intratumoral distribution of [¹⁴C]thymidine was visualized and evaluated semiquantitatively by autoradiography using a phosphor imager. **Results:** In both groups, tumor volume decreased until day 7 after irradiation; afterward, regrowth occurred in only the group that had received 6 Gy. A decrease in thymidine uptake was found as early as 8 h after irradiation. On day 3 after irradiation, thymidine uptake increased again in the 6-Gy group, before the increase in tumor volume, but remained unchanged in the 20-Gy group. Also on day 3, multiple foci of thymidine uptake suggesting proliferation preceding tumor recurrence were seen on autoradiographs from the 6-Gy group but not from the 20-Gy group. Histological findings correlated with the results of autoradiography. **Conclusion:** The results show that radiolabeled thymidine is a sensitive tracer for assessment of early tumor response and recurrence after irradiation. The rapid decrease in uptake, however, does not allow any prediction about tumor recurrence.

Key Words: radiolabeled thymidine; tracer for tumor response; tracer for tumor recurrence; cell proliferation

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In vitro, ³H- or ¹⁴C-labeled thymidine has been widely used as a marker for observing cell proliferation and the effects of therapy. In vivo, however, the well-documented extensive catabolism of [¹⁴C]thymidine limits such use with PET (1,2). Therefore, using a xenotransplanted human colon carcinoma model, this study investigated the relationship between [¹⁴C]thymidine uptake by tumors after irradiation, the recurrence or inhibition of growth of tumors and the histology of tumors. Semiquantitative autoradiography with phosphor imaging was used to evaluate the distribution of

thymidine uptake within tumor sections over time after irradiation.

MATERIALS AND METHODS

Tumor Xenografts, Growth Curves and Irradiation

After receiving an anesthetic, nude mice bearing subcutaneous human SW707 colon carcinoma xenografts between 0.8 and 1 cm in diameter were exposed to single doses of 6- or 20-Gy photon irradiation using a clinical therapy unit. At the same time, control animals were anesthetized and sham irradiated.

Tumor diameters in two perpendicular axes were determined with a caliper 1 and 2 d before and daily up to 14 d after irradiation, and growth curves were established as changes in mean volume over time relative to volume on the day of irradiation.

Tumor Uptake of [¹⁴C]Thymidine

At 4, 8 and 24 h and at 2, 3, 4, 7, 10 and 14 d after irradiation, mice were injected intravenously with 185 kBq [2-methyl-¹⁴C]thymidine (specific activity 2.04 GBq/nmol).

Six animals per group and per time point were killed by heart puncture 60 min after injection, and the radioactivity in the tumor was measured with a liquid scintillation counter. At 8 h and 3, 7, 10 and 14 d after irradiation, half of the tumor was cut into 10-μm sections. Distribution of activity within the slices was evaluated semiquantitatively with a phosphor imager in various regions of interest (ROIs). For 10 d, the sections of the tumor were placed in close contact with imaging plates composed of fine crystals of BaFBr:Eu²⁺ in an organic binder. The digital image was viewed on a video monitor and analyzed with the ROI technique. The data were expressed as counts per pixel. For histological evaluation, tumor sections were stained with hematoxylin and eosin.

RESULTS

Tumor Growth Curves

Figure 1 shows the tumor volumes of control and irradiated mice 2 d before and 14 d after irradiation. Both irradiated groups had the same slope for volume decrease until 7 d after irradiation. Tumor regrowth was found after 7 d in the 6-Gy group. At day 10, the increase in volume paralleled that of the control group.

No regrowth was observed in the 20-Gy group. Tumor volume remained the same between days 7 and 14 after irradiation.

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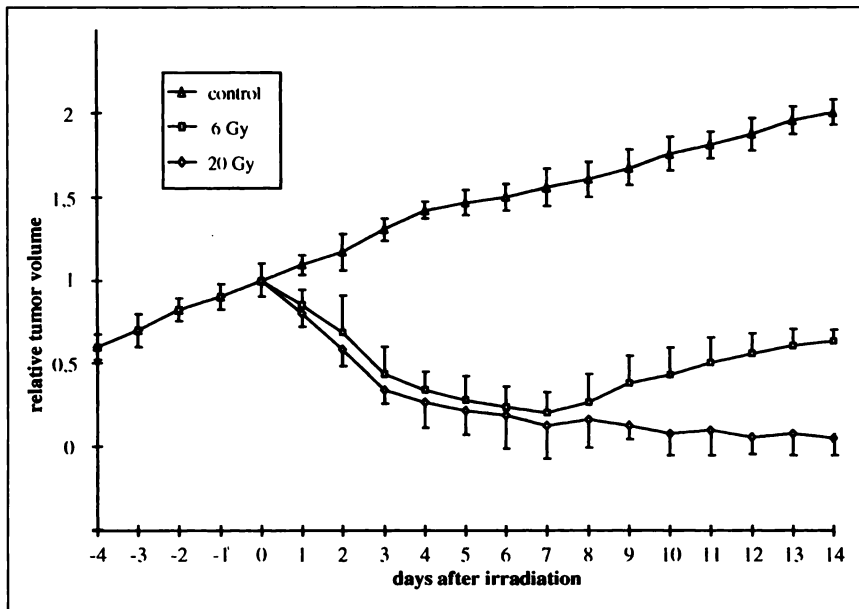


FIGURE 1. Tumor volume changes in SW707 control tumors and SW707 tumors exposed to 6 or 20 Gy, relative to tumor volume on day of irradiation (mean \pm SD, $n = 10$).

Tumor Uptake of [¹⁴C]Thymidine

After irradiation of tumors with 6 or 20 Gy, the activity concentration decreased rapidly within 8 h and then remained nearly unchanged until 48 h (Fig. 2). A slight increase in uptake was observed in the 6-Gy group on day 3 and was followed by further increases until day 14. Currently, the activity in the irradiated tumors exceeds that in the control tumors. The increase in uptake after 6-Gy irradiation preceded an increase in tumor regrowth by approximately 4 d ($P < 0.05$ from day 7). The low uptake until day 14 after 20-Gy irradiation correlated with a lack of tumor regrowth. The decrease in uptake after irradiation, however, was the same in both groups regardless of later tumor regrowth.

Autoradiography and Histology

Table 1 summarizes the results of semiquantitative evaluation of [¹⁴C]thymidine uptake in various tumor slices after evaluation of the phosphor imager autoradiographs. Images of untreated tumors clearly show heterogeneous activity distribution (Fig. 3A). At 8 h after irradiation, a low thymidine uptake in the entire tumor section was found for both groups (Fig. 3B).

Three days after a 6-Gy exposure, several single foci of thymidine uptake were seen, indicating increased activity (Fig. 3C). On days 7 and 10, multiple foci of uptake were seen in correlation with an increase in tumor regrowth (Figs. 3D and E). On day 14, the mean uptake was higher than in control slices (Fig. 3F). After 20-Gy irradiation, the uptake

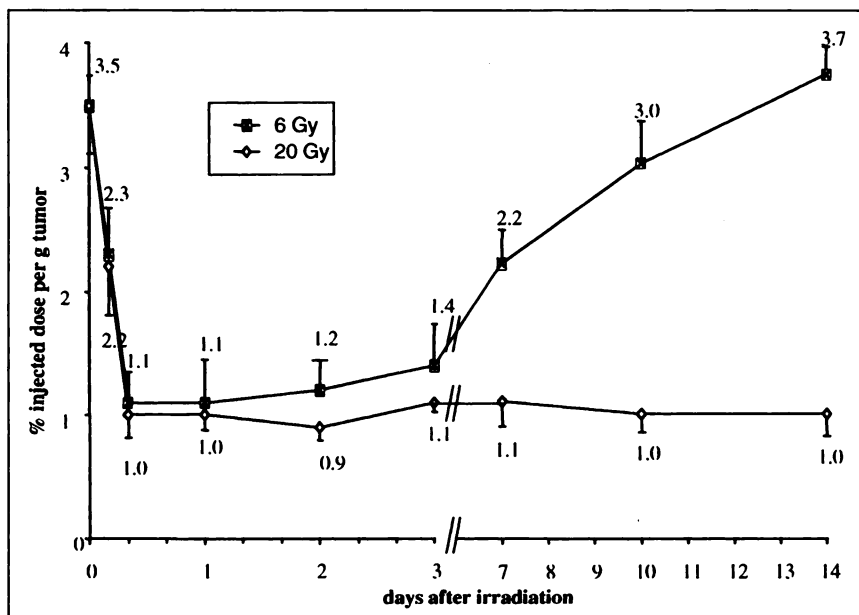


FIGURE 2. Time course of [¹⁴C]thymidine uptake in tumor tissue after exposure to 6 or 20 Gy in % injected dose per gram of tissue (mean \pm SD, $n = 6$).

TABLE 1
Autoradiographic Results in Tumor Sections Before and at Different Time Points After 6-Gy Irradiation, Obtained from Phosphor Imager

Time	Mean counts per pixel (range)
Before irradiation	31.8 (16.6–72.5)
8 h after irradiation	9.4 (7.8–11.2)
3 d after irradiation	12.7 (8.1–26.3)
7 d after irradiation	18.7 (8.3–38.4)
10 d after irradiation	27.4 (12.7–62.7)
14 d after irradiation	34.2 (25.3–71.4)

remained at the low, 8-h, level. These semiquantitative results correspond to the quantitative tissue measurements.

The wide range in thymidine uptake found by autoradiography of control tumors cannot be explained histologically. Slices obtained 3 d after 20-Gy irradiation show large areas of degenerating tissue. Slices obtained 10 d after 6-Gy irradiation show, in addition to pyknotic cells and connective tissue, many vital tumor cells. Slices obtained 10 d after 20-Gy irradiation show large amounts of fibrotic tissue but no vital tumor cells.

DISCUSSION

Uptake of radiolabeled nucleotide precursors is widely used as an index of tissue proliferation. PET with [¹¹C]thymidine has potential for noninvasively measuring the proliferative capacity of tumors in vivo and for assessing the response to therapy. In vivo, however, quantification is limited by the extensive catabolism of thymidine. Within several minutes after injection of thymidine, high-performance liquid chromatography of blood revealed rapid conversion to thymine and to other metabolites, such as dihydrothymine (1,2).

Despite this phenomenon, our xenotransplanted human colon carcinoma model responded rapidly to a single dose of

only 6 Gy, as shown by significantly reduced thymidine uptake before tumor reduction and increased uptake on day 3 before tumor regrowth. Thymidine uptake in the 20-Gy group remained low until day 14. Autoradiographs obtained with a phosphor imager could show this time course for thymidine uptake.

Thymidine uptake in control tumors varied significantly—by a factor of 4.5—within slices, with no histological correlation. This finding indicates a marked variation in biochemical activity within tumors, as has already been shown experimentally (3).

After irradiation, solid tumors undergo different volume responses. After exposure to sublethal radiation, tumors may continue to grow briefly and later shrink as the tumor cells are devitalized, lysed and resorbed. At the same time, surviving cells whose division was arrested at the transition between the G₂ phase and mitosis (G₂M block) begin to proliferate and repopulate the tumor (4,5). This phenomenon was seen in our experiment from the thymidine uptake in small hot spots. Tumor volume and thymidine uptake are, therefore, a result of depopulation and repopulation of tumor cells after irradiation. After exposure of our model to 6 Gy, cell division was completely inhibited for 3 d; after exposure to 20 Gy, however, all tumor cells were devitalized and showed no signs of repopulation.

Tumor volume is also affected by host blood cells and invasion by fibroblasts. Fibroblasts are responsible for the large amount of fibrotic tissue found in tumors after irradiation. Therefore, one can conclude that tumor volume, the parameter most frequently used to assess response to irradiation, can show only small changes. In these experiments, the remaining tumor tissue after exposure to 20 Gy was entirely fibrotic.

CONCLUSION

Despite the extensive catabolism of radiolabeled thymidine in vivo, it is useful for imaging tumor proliferation after

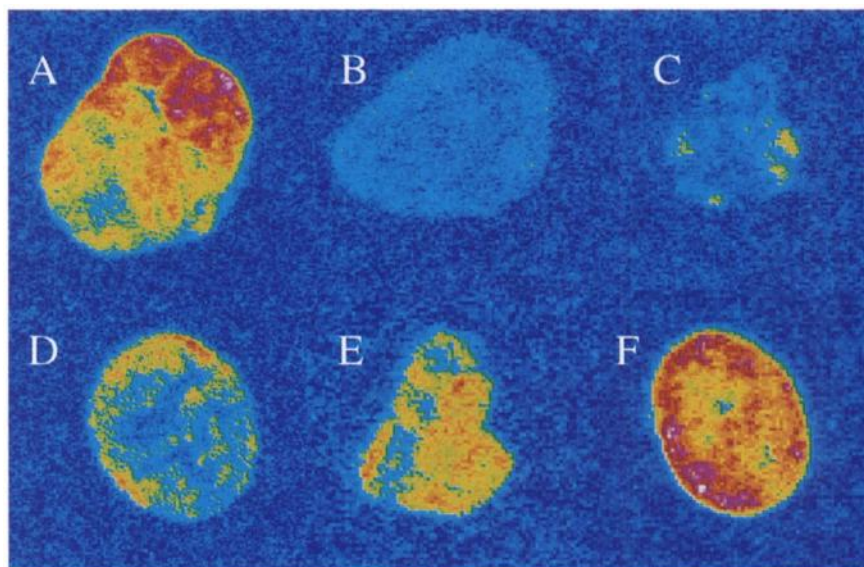


FIGURE 3. Autoradiographs of [¹⁴C]thymidine distribution within tumor slices before (A) irradiation with 6 Gy and 8 h (B), 3 d (C), 7 d (D), 10 d (E) and 14 d (F) afterward.

irradiation. A correlation was found between thymidine uptake and the regrowth or inhibition of growth of tumors.

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