Rapid and Sensitive Response of Carbon-11-L-Methionine Tumor Uptake to Irradiation

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Evaluation of cancer treatments by the measurement of tumor size is an unsatisfactory method for the observation of the radiobiologic response of the tumor. After 20 Gy single-dose irradiation of ^{60}Co to rat tumor AH109A, the L-[methyl-^{11}C] methionine tumor uptake, the microscopic extension of tumor necrosis and the shrinkage of tumor were compared quantitatively. L-[Methyl-^{11}C]methionine uptake fell to 54 ± 19% of non irradiated tumor at 12 hr after irradiation. Necrosis extended 49 ± 7% of total tissue volume after 3 days. Tumor volume decreased 48 ± 12% 10 days after irradiation. L-[Methyl-^{11}C]methionine uptake by tumor showed a sharp and rapid linear decrease after irradiation and the response of the uptake to irradiation preceded the extension of necrosis and tumor shrinkage. We conclude that radiation effect on the tumor may be evaluated immediately after irradiation by the measurement of L-[methyl-^{11}C]methionine uptake and thus clinical application of positron emission tomography may give a benefit to the patients undergoing radiotherapy.

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Determination of radiobiologic effects on the tumor in vivo, such as growth delay (*1*) and lung colony assay (*2*), are ideal but not applicable to the clinic, and the measurement of tumor size (*3*) is conventional but unsatisfactory in correlating the radiation effect with cell damage in metabolic and biological terms. Development of a new technique is needed.

Uncontrolled malignant cell proliferation requires increased metabolic activity. Studies of accumulation of carbon-11- (^{11}C) labeled amino acids demonstrate a markedly elevated incorporation into tumor tissue (*4*), and provide a method for early detection and grading of lung cancer by positron emission tomography (PET) using L-[methyl^{11}C]methionine ([^{11}C]Met) (*5–7*). Also amino acid transport and metabolism have high radiosensitivity (*8*). These studies suggest that [^{11}C]Met accumulation may reflect the changes of the metabolism and viability of tumor under radiotherapy. The present study aims to establish a new indicator which not only monitors the biologic response of irradiated tumors but is desirably open to clinical application, where the [^{11}C]Met tumor uptake, micromorphometry of tumor necrosis, and tumor volume are correlated after experimental radiotherapy of rat AH109A tumor.

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MATERIALS AND METHODS

The research described in this report, involving animals maintained in the animal care facility of our Institute, was fully accredited by the laboratory animal care committee of Tohoku University.

Tumor, Rats, and ^{60}Co Irradiation

A 0.1 ml of suspension containing 7×10^6 cells of ascitic hepatoma AH109A was subcutaneously inoculated in a thigh of young male Donryu rats weighing from 180 to 250 g. Irradiation was performed when a transplanted tumor grew to 1.5 to 2.0 cm in diameter. A rat was anesthetized with 10 mg i.p. of sodium pentobarbital, then fixed with adhesive tapes so as to place the tumor-bearing thigh in the field of irradiation. Thus, the other parts of body were left outside the field, though shields were not used (Fig. 1). The tumor was exposed to 20 Gy of single-dose cobalt-60 (^{60}Co) irradiation at a dose rate of 0.5 Gy/min at 65 cm SSD and 1 cm depth with a 5-mm sheet of bolus (tissue equivalent material).

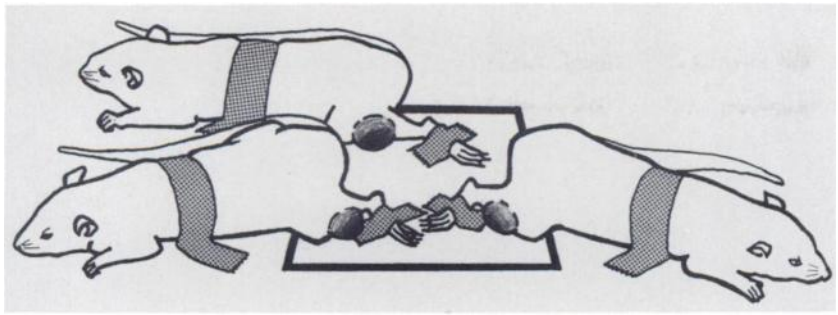

FIGURE 1
Illustration of the irradiation to the AH109A tumor in the rat thigh. Radiation field of ^{60}Co was rectangular area.

Tumor Growth Study

Solid tumor on the thigh was measured with a vernier caliper everyday during the experiment. The product of the three principal diameters of the tumor was designated as "tumor volume" which, in a previous study (*9*), proved to have a linear correlation to the actual volume of excised tumor. Two groups of eight rats each were used for tumor growth study with or without irradiation.

Carbon-11-Met Uptake Study

Five groups, each containing 8 to 20 rats, were administered [^{11}C]Met, 6, 12, 24, 72, and 144 hr after the irradiation, and one group without irradiation was used as a control.

Carbon-11-Met was synthesized as described previously (*5,10*). The radiochemical purity was over 99%. After fasting for 8 hr, ~100 μCi of [^{11}C]Met was intravenously 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), as

$$DUR = \frac{\text{counts of tissue/sample weight}}{\text{injection dose counts/body weight}}.$$

Morphometry

Tumor samples obtained at 1, 3 and 6 days after irradiation and those from nonirradiated controls were processed for quantitative histopathologic studies. Microscopic samples, sectioned at 3 μm, were stained with HE, Elastica-Goldner, and Mallory's Azan stains. In these, the ratio of the necrotic to the total tissue volume (Vn) was estimated by measuring the corresponding ratio in the sectional area (An). This was performed by line sampling on microscopic section (*11*): parallel test lines were set on a microscope slide at an interval of 1 mm, which, by crossing the necrotic and the non-necrotic areas, produced a number of intercepts with length λ_n from the former, and λ_{nn} from the latter areas. The lengths of the individual intercepts were measured sequentially under a microscope, so that the percent of necrosis versus the total area of tumor was calculated by

$$Vn = An = \Sigma\lambda_n/(\Sigma\lambda_n + \Sigma\lambda_{nn}).$$

In each rat, morphometry was performed on three samples of tumor.

RESULTS

Figure 2 shows the effect of radiation on the growth of the tumor. Even after a single dose 20 Gy of ^{60}Co irradiation, tumors continued to enlarge until the next day, then began to shrink. On Day 6, the volume became significantly smaller than at the time of irradiation (76 ± 16%, $p < 0.05$); on Day 10, it was reduced to half (48 ± 12%, $p < 0.001$). This was followed by a shrinkage of tumor, without showing any sign of regrowth (not shown in the figure).

Figure 3 shows the [^{11}C]Met uptakes by the tumor, the femur from the tumor-bearing thigh, the muscles from the contralateral thigh, and the peripheral blood, sequentially after irradiation. The nonirradiated group is plotted at time 0. The figure demonstrates that the [^{11}C]Met uptake by the tumor drops sharply at 6 and 12 hr after irradiation, down to a level of 54 ± 19% ($p < 0.001$) of the nonirradiated group, then decreases gradually to 30 ± 5% ($p < 0.001$) on Day 6. The uptake by the femur with bone marrow also decreased until Day 3 (58 ± 5%, $p < 0.001$), but there was a slight recovery to 69 ± 11% at Day 6 with a significance of p

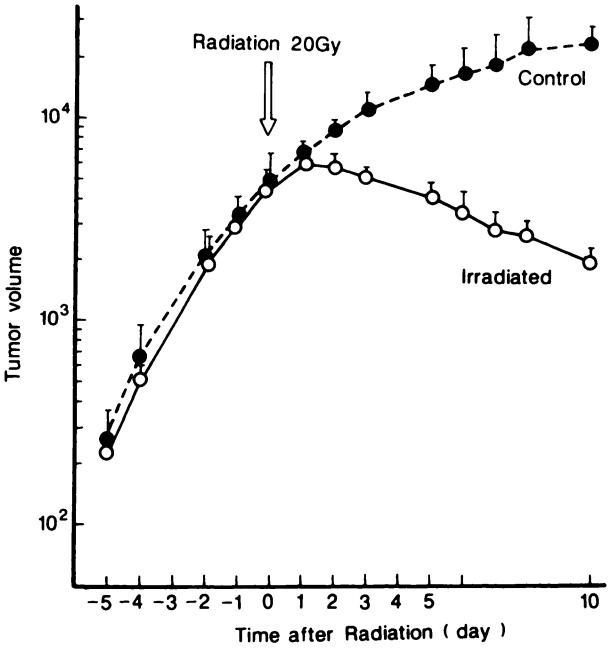

FIGURE 2
Tumor growth curve of AH109A showed the effect of irradiation. Ordinate: product of the three principal diameter of the tumor (cu mm), logarithm plots.

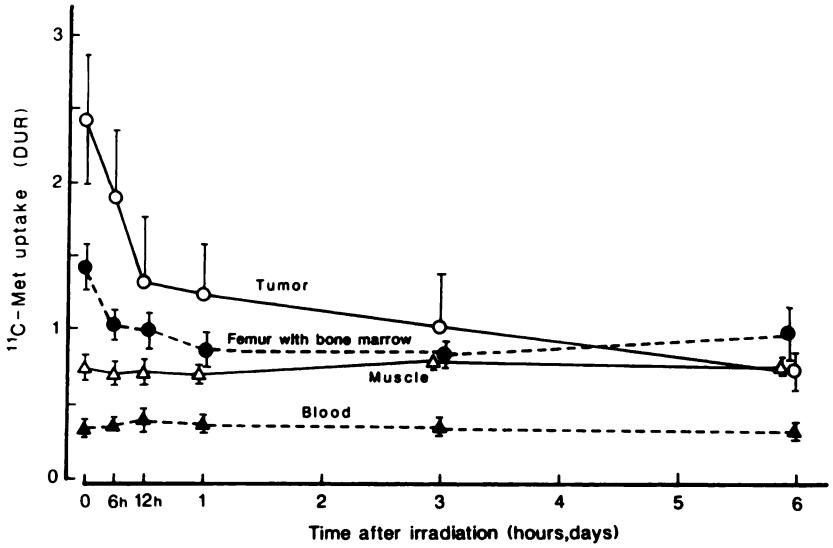

FIGURE 3
Effects of 20 Gy single-dose ^{60}Co irradiation on the tumor and other tissue uptakes of [^{11}C]Met 30 min after injection.

< 0.01 compared to Day 3. The blood level and the uptake by the muscles remained low and constant.

In quantitative histopathologic studies, the nonirradiated tumors contained 12.6 ± 5.4% of necrotic tissue (Fig. 4). In the irradiated tumors, the percentage of necrotic area increased remarkably thereafter, to 49.0 ± 6.6% ($p < 0.001$) on Day 3 and up to 68.6 ± 7.4% ($p < 0.001$) on Day 6.

Comparative analysis was made in Figure 5, to establish the sequential relationship between the [^{11}C]Met uptake by tumor, the percentage of necrosis and the tumor volume. In the irradiated tumors, the reduction of [^{11}C]Met uptake occurred first, followed by the increase of necrotic area, and in the last place came the reduction of tumor volume. A reduction by one-half took 12 hr for [^{11}C]Met uptake and 10 days for tumor volume, while necrotic area extended to one-half of total tissue at 3 days.

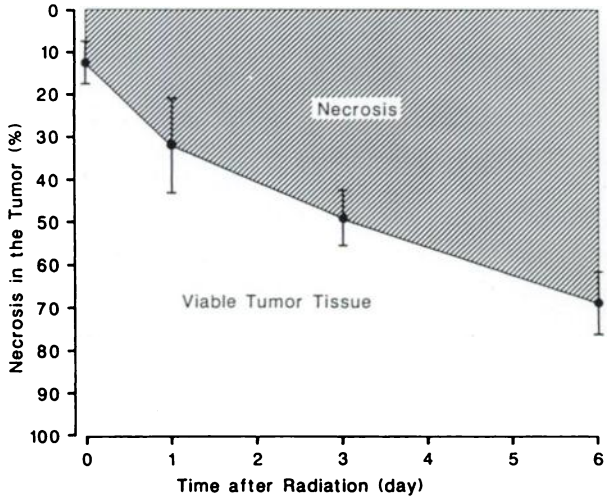

FIGURE 4
Effect of radiation on the tumor necrosis of AH109A. Results of quantitative morphometrical study.

DISCUSSION

Amino acid accumulation in tumor cells is mediated by the tumor blood flow (*12*), active transport at the cell membrane (*13*) and the increased metabolic requirement by the cell itself (*14*). The cell membrane, with its high sensitivity to radiation, is one of the major targets of radiation injury (*15*). Radiation injures the membrane transport system for amino acids (*8*). Deterioration of various important enzymes also occurs at an irradiation of 20 Gy (*16*). In view of this, the reduced [^{11}C]Met uptake by the tumor is likely to reflect damage to the membrane transport system or inactivation of enzymes for amino acid metabolism, or both.

The radiation triggers a series of degenerative and cytolytic changes, finally leading to necrosis of cells (*17*). Since, microscopically, it is impossible to determine whether a cell under the degenerative process still possesses a potential of proliferation (*18*), we employed necrosis as the definite expression of cell death. Thus, in our micromorphometry technique we assess the extension of radiation damage in accurate quantitative terms, while previously, it has been subject to a rather arbitrary evaluation by "grading." It is not certain what metabolic changes cause the cell damage. However, it has been demonstrated that the reduction of [^{11}C]Met uptake precedes any visible extension of necrotic areas.

The tumor growth depends on the balance between the cell production and cell loss (*19*), and the tumors with lower cell loss continue to grow for a couple of days after irradiation and then start a gradual shrinkage (*20*). Thus, the tumor volume can be influenced by either edematous swelling or collapsing of dying tissues. The shrinkage of tumor which is dependent on the elimination of the dead cells occurs after the development of necrosis.

Dependence of the [^{11}C]Met uptake by tumor upon radiation dose was also observed in a separate experi-

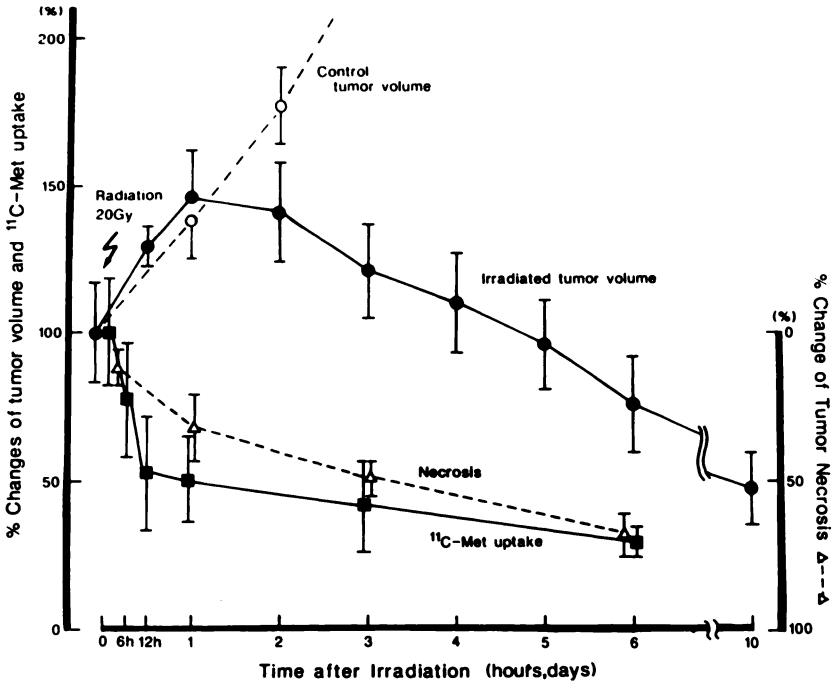

FIGURE 5
Comparison of the tumor volume, morphometry and [^{11}C]Met tumor uptake.

ment. After an irradiation of 10 Gy, the uptake of [^{11}C] Met and the reduction of tumor volume showed the same tendency as above, while it was followed by a regrowth of tumor associated with a parallel increase of uptake.

The bone marrow, besides its well-known radiosensitivity, proved to be capable of rapid regeneration, reaching the peak 7 days after irradiation (*21*). The change with time of the [^{11}C]Met uptake by the femur with bone marrow is fully consistent with these radiobiologic phenomena.

The correlation of tracer uptake to the irradiated tumor has been demonstrated with fluorine-18-2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG) (*22*) and with nitrogen-13-glutamate (*23*). However, nothing has been known about the discrepancy between the volume and the histopathologic changes of tumor. The correlation of glucose metabolism to amino acid metabolism after the irradiation has not been studied well. But ^{11}C- or [^{14}C]Met seems to be more susceptible than FDG to the irradiation from our preliminary double tracer study.

Irradiation produces free radicals, which induce injuries to enzymes, membranes, and DNA. Thus, altered metabolism involved in amino acid reflected by [^{11}C] Met uptake precedes and leads to the death of cell observed as necrosis. The visible reduction of tumor volume does not reveal itself until a larger part of the necrosis has been removed.

CONCLUSION

Our study suggests that [^{11}C]Met uptake expresses the biologic response of tumor to radiotherapy much more sensitively than does the tumor volume reduction and the extension of necrosis. The therapeutic effect may be evaluated immediately after irradiation by the response of [^{11}C]Met uptake and it will give a great benefit to the patients on radiotherapy. Its clinical application using positron emission tomography is to be encouraged.

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