Radiation-Induced Inhibition of Tumor Growth as Monitored by PET Using L-[1-^{11}C]Tyrosine and Fluorine-18-Fluorodeoxyglucose

Bernard J. G. Daemen, Philip H. Elsinga, Anne M. J. Paans, Andre R. Wieringa, Antonius W. T. Konings, and Willem Vaalburg

PET Center, University Hospital and Department of Radiology, State University, Groningen, The Netherlands

The potential use of PET to monitor radiotherapeutic effects on tumors has been evaluated with L-[1-^{11}C]tyrosine and ^{18}FDG. Single x-ray doses of 10, 30, or 50 Gy have been applied to rhabdomyosarcoma tumors growing in the flank of rats. Dose-dependent reductions of tracer uptake were registered by PET 4 and 12 days after treatment. These later effects on tracer uptake appeared to correlate with changes in tumor volume. Therefore, PET using L-[1-^{11}C]tyrosine and ^{18}FDG is suitable to monitor kinetics of tumor growth and tumor regression after radiotherapy. Direct effect on tracer uptake was not observed within 8 hr after irradiation. This indicates that, using PET, early predictions on the outcome of radiotherapy are not possible. When combining a radiation treatment with hyperthermia, radiation-induced inhibition of tumor growth was clearly enhanced. Tracer uptake remained at the pretreatment value, possibly due to invasion of host cells. From these experiments, it can be concluded that it is difficult to monitor a combined treatment of radiation and hyperthermia by PET.

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In clinical practice, it is difficult to give a reliable prognosis concerning the curative outcome of a radiotherapeutic treatment. With positron emission tomography (PET), metabolic processes in tissues, such as synthesis of macromolecules and glycolysis, can be investigated and quantified. Therefore, PET might be an elegant technique for monitoring tumor treatment on the basis of tumor metabolism.

Irradiation of tumors is known to result in inhibition of DNA synthesis, whereas protein synthesis and carbohydrate metabolism are only marginally influenced at the doses used in clinical practice (*1–4*). Direct radiation effects on glycolysis, protein synthesis or amino acid transport can only be expected at high doses (>100 Gy) (*5–7*). From this, it may be expected that PET measurements, used to monitor acute radiation effects on protein synthesis and glycolysis, are less profitable for prognosis of radiation effects on tumor growth.

In rats, Knapp et al. (*8*) measured acute reductions of ^{13}N-glutamate uptake into Walker 256 carcinosarcomas 30 min after 8 Gy ^{60}Co-irradiation. Also in tumor-bearing rats, Kubota et al. (*9*) found a relatively rapid decrease of L-[methyl-^{11}C]methionine uptake within 6 hr after 20 Gy ^{60}Co irradiation. This prompted us to evaluate, in an experimental animal model, direct and indirect radiation-induced changes of amino acid utilization of tumors by PET. To correlate amino acid uptake with protein synthesis in the tumor tissue, parallel studies with ^{14}C-labeled amino acids are necessary in which the percentages of amino acid incorporated into proteins are determined.

It is difficult to determine the actual tumor volume in patients undergoing radiotherapy. In clinical studies with 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}FDG), PET data were merely correlated with the clinical outcome of treatment, but not with the tumor volume present at the time of the PET measurement (*10–12*). It is therefore important to correlate PET data obtained in tumors before and after radiotherapy with the actual radiobiological effects on tumor growth at the time of PET.

Hyperthermia rapidly suppresses protein synthesis in tumor tissue (*13,14*). This phenomenon appeared to be useful for the prediction of tumor growth by PET after a hyperthermic treatment (*15*). Furthermore, hyperthermia has a sensitizing effect on treatment with ionizing radiation (*16,17*). It is therefore of interest to evaluate the effects of the combined treatment of radiation and hyperthermia on L-[1-^{11}C]tyrosine (^{11}C-tyr) and ^{18}FDG uptake and to correlate these data with the effects on tumor growth.

The aims of the current study are:

1. To investigate the effects of radiotherapy as well as the combination of radiotherapy and hyperthermia on tumor uptake of ^{11}C-tyr and ^{18}FDG with PET, and to compare the ^{11}C-tyr data with L-[1-^{14}C]tyrosine (^{14}C-tyr) data on uptake and incorporation as obtained after dissection of tumor.

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For reprints contact: W. Vaalburg, PET Center, University Hospital, Oostersingel 59, 9713 EZ Groningen, The Netherlands.

2. To determine the effects of the respective treatments on tumor growth and to correlate these with the PET data.

MATERIALS AND METHODS

Radiotracers

According to Bolster et al. (*18*), the radiopharmaceutical ^{11}C-tyr with a radiochemical purity of >99% and a specific activity of >3.7 GBq/μmol (100 Ci/mmol) was synthetized via the isocyanide route. ^{18}FDG was synthetized according to Hamacher et al. (*19*). The radiochemical purity of ^{18}FDG was >97%.

Animals

Rhabdomyosarcoma-bearing rats (*20*) were purchased from TNO, Rijswijk, The Netherlands. Cubic pieces of rhabdomyosarcoma tissue that weighed 100 mg were dissected from these animals and subcutaneously grafted into the left flank of 2-mo-old female Wag/Rij rats with a weight of 140 g. Eighteen days after transplantation, the tumors developed to ellipsoid-shaped volumes between 4 and 5 ml. At these volumes, the tumors were free of necrotic tissue. The rats had free access to water and food (RMH pellets, Hope Farms, Woerden, The Netherlands).

Radiotherapy and Hyperthermia

Prior to a radiotherapy, the tumor-bearing animal was sedated with an intraperitoneally injected dose of 1.5–2.0 mg sodium pentobarbital (1.5 g/100 ml saline) per 100 g body weight. The body of the rat was protected by a telescoping lead cylinder with a total shielding thickness of 4 mm. From these lead shields, the tumor protruded through a slit. For irradiation a Philips-Muller Mg 300 Röntgen source, operated at 200 kV and 15 mA, was used. Only the tumor was irradiated. X-rays were filtered with 0.5 mm Cu and 0.5 mm Al. The dose rate was 3 Gy/min. Each animal received a single dose of 10, 30 or 50 Gy, respectively. Ten minutes after irradiation, a number of the animals that were exposed to 30 Gy received a hyperthermia treatment at 45°C for 15 min. Hyperthermia was carried out as described previously (*15*).

L-[1-^{14}C]Tyrosine Experiments

At 8 hr, 4 days or 12 days after radiotherapy, rats with tumors exposed to doses of 10 Gy, 30 Gy or 50 Gy, were investigated with ^{14}C-tyr. At these points in time, rats were anaesthetized as described below and intravenously injected with 93 kBq (2.5 μCi) ^{14}C-tyr (specific activity 2 MBq/μmol, Amersham International Buckinghamshire, UK). Forty-five minutes after this injection, the rats were killed by a heart puncture, and the tumors were rapidly dissected. Seven samples (50–100 mg) were obtained from the dissected tumors, except from the tumors investigated 12 days after 10 Gy single-dose radiotherapy. In the latter case the volumes were larger than 10 ml, and necrosis amounted to 10% of the total tumor weight. From this necrotic tissue, four samples were taken as well. Each of the five tumor samples and the two necrosis samples were dissolved in 1.5 ml Protosol© (Dupont, Boston, MA) and after the addition of 10 ml Plasmasol© scintillation liquid (Packard Instruments, Downers Grove, IL), the ^{14}C-radioactivity of the samples was measured by liquid scintillation counting. The uptake of ^{14}C-tyr into the tissue was calculated as the differential absorption ratio (DAR), i.e. (activity sample/activity injected) × (weight rat/weight sample). The remaining two tumor samples and the two necrotic samples were used to determine the incorporation of ^{14}C-tyr into tumor proteins. These samples were homogenized and the protein fraction was precipitated with trichloro acetic acid. The acid insoluble fraction was expressed as a percentage of the total ^{14}C-activity of the tumor. Tumor tissues of eight untreated animals served as control.

PET Experiments

Animals were intraperitoneally anaesthetized with 3–4 mg sodium pentobarbital (3 g/100 ml saline) per 100 g body weight, and received a catheter temporarily inserted into a tail vein to facilitate complete injections of tracer. In order to sustain anesthesia, rats were given an additional dose of 1 mg per 2 hr. Anesthesia had a lowering effect on body temperature. To maintain body temperature within the physiological range, animals were irradiated with infra red lamps.

PET data were acquired with a stationary double-headed positron camera (*21*). This camera, with a resolution of 5.5 mm, has a sensitivity of 2.7 cps/KBq (100 cps/μCi). The accuracy of quantification, based on the statistical error in the measured counts amounted less than 2% for the tumor. The quantification with this system and this type of tumor-bearing animals has extensively been described by Daemen and co-workers (*22,23*). The time schedule for the consecutive PET measurements is given in Figure 1. After positioning the untreated rat into the PET camera, an intravenous dose of 1.1 MBq (30 μCi) ^{11}C-tyr in a volume of 0.2 ml was administered as a fast bolus, and PET data were acquired for 45 min. After each injection, the catheter was flushed with 0.05 ml saline. Three hours after the ^{11}C-tyr study, in the same tumor-bearing rat, a second PET study was performed with 1.1 MBq (30 μCi) ^{18}FDG for 45 min. After regaining consciousness, the rat was allowed to recuperate for 1 day and was then subjected to radiotherapeutic and hyperthermic treatment. Eight hours after these treatments, the tumor-bearing rat was monitored with ^{11}C-tyr as described above. Twelve hours after treatment, when ^{11}C-activity was reduced to a negligible background level, the animal was also investigated with ^{18}FDG. Identical PET studies with ^{18}FDG and ^{11}C-tyr were carried out 4 days and 12 days after treatment. At the end of experimentation, each individual animal had undergone a total of eight scans, four with ^{11}C-tyr and four with ^{18}FDG. The radioactivity uptake into the tumor was calculated from the measured counts in a 5-min time frame between 40 and 45 min after injection, and expressed as a DAR, i.e., DARPET: (counts tumor/volume tumor) × (weight animal/counts animal).

Growth Curves and Growth Delays

The three principal diameters of the tumors were measured in millimeters with vernier calipers while the animal was under ether anaesthesia. From these measured diameters, the volumes of the ellipsoid-shaped tumors were calculated using the formula: $V = \frac{1}{6}\pi \times \text{length} \times \text{width} \times \text{thickness}$ (*24*). These data were plotted as growth curves and the tumor doubling times (TD) were calculated. TD is the time needed for a tumor to double its treatment volume. The treatment effect on tumor volume can be expressed as growth delay (GD), which is calculated with the formula: $GD = (TD_{tr} - TD_c)/TD_c$, in which TD_{tr} is the doubling time for the treated tumors, and TD_c is the doubling time obtained in a control group of untreated animals. Statistical significance was analyzed with Fisher's distribution free sign test (*25*).

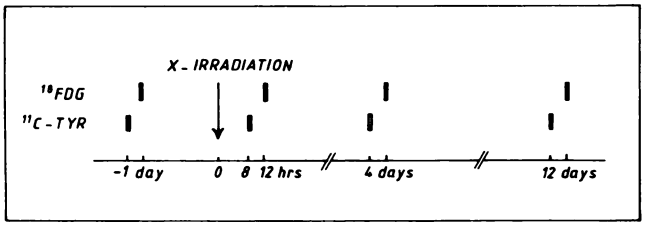

FIGURE 1. Time schedule of the experimental procedure for PET measurements. Carbon-11-tyr (□) and ^{18}FDG (■) data were acquired for 45 min. Time interval between ^{11}C-Tyr and ^{18}FDG studies is 4 hr.

RESULTS

PET Experiments

PET images, as presented in Figure 2, show the distributions of ^{11}C-tyr and ^{18}FDG in a Wag/Rij rat with a rhabdomyosarcoma. In panels A and B, the uptake of ^{11}C-tyr and ^{18}FDG into untreated tumors is shown at 45 min after injection. The tumors are indicated with a horizontal line. In panels C and D, distributions of ^{11}C-tyr and ^{18}FDG at 12 days after 50 Gy irradiation are shown. From these images, it can be observed that the tumor volume as well as the amount of radioactivity per unit of volume is decreased after treatment.

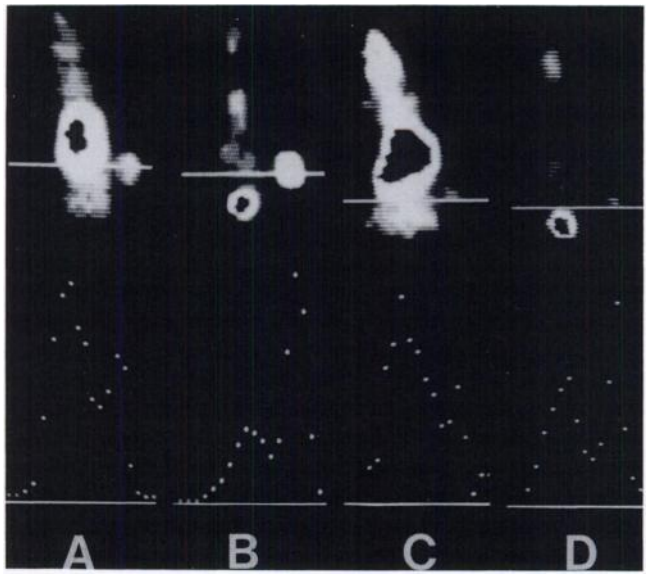

FIGURE 2. Distribution of ^{11}C-tyr (A) and ^{18}FDG (B) as measured by PET at 45 min after intravenous injection into the same untreated RMS-bearing rat. Corresponding distribution of ^{11}C-tyr (C) and ^{18}FDG (D) acquired 12 days after 50 Gy irradiation. Tumors are indicated by horizontal lines. Black areas in the images are caused by windowing in order to obtain clearer visualization of the tumor.

The uptake values of ^{11}C-tyr and ^{18}FDG into rhabdomyosarcoma tissue were calculated as DARPET (Table 1). The average DARPET values for ^{11}C-tyr and ^{18}FDG of 28 untreated tumors were 1.59 and 3.49, respectively. From these values, an ^{18}FDG/^{11}C-tyr uptake ratio of about 2 was calculated. This ratio was observed at all points of time

TABLE 1
PET Measurements on Uptake of ^{11}C-tyr and ^{18}FDG into Tumor Tissue of Rhabdomyosarcoma-bearing Wag/Rij Rats Before and After Radiotherapy Only and Radiotherapy Combined with Hyperthermia

Treatment	Tracer	Before	Time after treatment			number
			8/12 hr†	4 days	12 days	
10 Gy	^{11}C-tyr	1.63 ± 0.17	1.78 ± 0.16	1.64 ± 0.18	1.61 ± 0.12	n = 8
		(100 ± 0)	(107 ± 5)	(99 ± 8)	(100 ± 4)	
	^{18}FDG	3.43 ± 0.21	3.76 ± 0.27	3.53 ± 0.39	3.73 ± 0.30	
		(100 ± 0)	(110 ± 7)	(103 ± 13)	(110± 10)	
30 Gy	^{11}C-tyr	1.70 ± 0.13	1.52 ± 0.12	1.28 ± 0.13*	1.89 ± 0.25	n = 8
		(100 ± 0)	(92 ± 9)	(79 ± 5)	(113 ± 14)	
	^{18}FDG	3.74 ± 0.27	3.73 ± 0.23	2.57 ± 0.27*	3.51 ± 0.49‡	
		(100 ± 0)	(103 ± 7)	(64 ± 10)	(96 ± 10)‡	
50 Gy	^{11}C-tyr	1.60 ± 0.06	1.57 ± 0.11	1.36 ± 0.15*	0.94 ± 0.08*	n = 7
		(100 ± 0)	(98 ± 4)	(83 ± 8)	(58 ± 3)	
	^{18}FDG	3.84 ± 0.29	3.64 ± 0.26	2.84 ± 0.32*	2.08 ± 0.27*	
		(100 ± 0)	(102 ± 10)	(79 ± 12)	(55 ± 8)	
30 Gy and HT§	^{11}C-tyr	1.35 ± 0.07	1.34 ± 0.13	1.48 ± 0.17	1.41 ± 0.15	n = 5
		(100 ± 0)	(100 ± 11)	(113 ± 17)	(107 ± 13)	
	^{18}FDG	2.72 ± 0.17	2.62 ± 0.50	2.82 ± 0.03	3.02 ± 0.43	
		(100 ± 0)	(103 ± 25)	(111 ± 23)	(120 ± 31)	

Uptake values expressed as DARPET; numbers in brackets are percentages.
Values are mean ± s.e.m.
* p values of 0.05 or less with paired Student's t-test for differences with the untreated situation.
† 8 hr for ^{11}C-tyr, 12 hr for ^{18}FDG.
‡ For this value n = 7.
§ HT = hyperthermia for 15 min at 45°C.

after the treatments. Consequently, the relative changes in uptake for both tracers are about the same, and therefore only results for ^{11}C-tyr are described.

It was observed that the relative uptake of ^{11}C-tyr into the 10-Gy exposed tumors was not changed after treatment. Four days after the 30- and 50-Gy treatments, reductions of about 20% were measured. After 12 days, the tumors treated with 30 Gy showed complete restoration of ^{11}C-tyr uptake to the level of the untreated situation, whereas the 50-Gy irradiated tumors showed further decline in ^{11}C-tyr uptake.

The combined treatment of 30 Gy x-radiation with hyperthermia did not have any significant effect on the relative ^{11}C-tyr uptake. Four days after treatment, a statistically significant difference in tracer uptake was observed between the tumors treated with 30 Gy only and the tumors treated with 30 Gy in combination with hyperthermia.

L-[1-^{14}C]Tyrosine Studies

Wag/Rij rats with untreated tumors and with tumors exposed to 10, 30 or 50 Gy were injected with ^{14}C-tyr. For reasons of comparison, the ^{14}C-tyr assays were carried out at the same points of time as in the PET studies using ^{11}C-tyr, namely 8 hr, 4 and 12 days after irradiation. Uptake of ^{14}C-tyr and incorporation of ^{14}C-radioactivity into proteins were measured in dissected tumor tissue obtained 45 min after injection. Total uptake was calculated as DAR. The incorporation into proteins was calculated as percentage of the amount of accumulated ^{14}C-activity (Table 2). All tissue samples were homogeneous and appeared to be representative for the total tumor, except at 12 days after 10 Gy, when a clear distinction could be made between areas with necrotic and vital tissue, which was histologically verified in microscopic slices. In general, the ^{14}C-uptake data obtained after dissection of the tumor tally with the ^{11}C-tyr uptake data as measured by PET (see Table 1). The amount of ^{14}C-tyr in the tissue with necrosis (N; last column in Table 2), measured at 12 days after 10 Gy irradiation, was about half the value of the untreated tumors. It is also notable that pieces of necrotic tissue have much lower amounts of ^{14}C-tyr than the vital parts (V) of the tumors. Since the volume of necrotic tissue is about 10% of the total, it is estimated that the total ^{14}C-tyr uptake, as expressed as DAR, is about 1.7. Therefore, the small amount of necrotic tissue has no significant effect on the total ^{14}C-tyr uptake or the total ^{11}C-tyr uptake.

At 4 days after irradiation with 10, 30 or 50 Gy, dose-dependent reductions of ^{14}C-tyr uptake of 21% ($p < 0.05$), 33% ($p < 0.01$) and 43% ($p < 0.001$) respectively, were observed. Twelve days after treatment the uptake of ^{14}C-tyr into tumors exposed to 10 and 30 Gy was restored to the value of the control group, while in the 50-Gy irradiated tumors, uptake was still significantly reduced with 35%.

The percentage of ^{14}C-tyr incorporated into proteins is considered to be a reflection of the protein synthesis in the tumor tissue. The incorporation values measured at different points of time after the respective irradiations (68%–87%) did not differ much from the values measured in the untreated situation (78%).

Growth Curves and Growth Delays

The effects of irradiation on tumor growth were evaluated by measuring tumor volumes in time course. To compare the effects of the respective irradiation doses on tumor growth, tumor volumes measured after treatment were normalized to the volumes at the time of treatment (100%). The data on relative tumor volumes were plotted to obtain the growth curves as shown in Figure 3. This figure shows that for a duration of 7 days after 10 Gy irradiation, tumor growth had stopped. During this period, the tumors that were exposed to 30 and 50 Gy showed decreasing volumes. After 12 days, the 10-Gy as well as the 30-Gy irradiated tumors were in progressive growth, in contrast to the 50-Gy tumors. Recurrence of growth of

TABLE 2
Uptake of ^{14}C-tyr into Tumor Tissue and Its Incorporation into Tumor Proteins of Rhabdomyosarcoma-bearing Rats After Radiotherapeutic Treatments at Different Time Points

		Time after radiotherapy		
Treatment	0 hr (n = 8)	8 hr (n = 5)	4 days (n = 6)	12 days (n = 5)
Untreated	1.86 ± 0.18*	—	—	—
	(78 ± 6)	—	—	—
10 Gy	—	2.11 ± 0.37	1.47 ± 0.31	V. 1.76 ± 0.12†
	—	(80 ± 6)	(73 ± 3)	(87 ± 4)
				N. 0.85 ± 0.40‡
				(82 ± 3)
30 Gy	—	1.68 ± 0.14	1.24 ± 0.29	2.01 ± 0.34
	—	(80 ± 4)	(73 ± 8)	(82 ± 3)
50 Gy	—	1.68 ± 0.21	1.07 ± 0.3	1.22 ± 0.10
	—	(77 ± 4)	(68 ± 8)	(78 ± 5)

* Uptake of ^{14}C-Tyr is expressed as DAR and incorporation of accumulated ^{14}C-Tyr activity as percentage (in brackets). Values are mean ± s.d.

† V = vital tissue and N = tissue with growth necrosis.

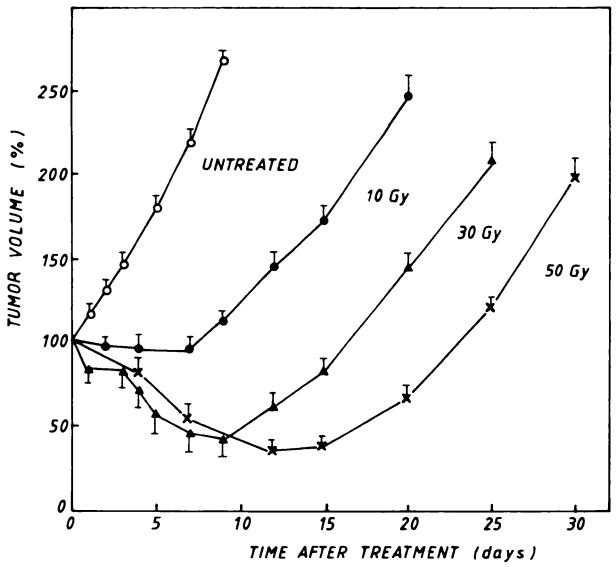

FIGURE 3. Growth curves of rhabdomyosarcoma tumors exposed to doses of 10 Gy (●, n = 8), 30 Gy (▲, n = 6) and 50 Gy (X, n = 6) and untreated tumors (O, n = 9). The measured volumes (mean ± s.e.m.) are normalized to the value at the time of treatment (100%). The mean doubling times for 10 Gy, 30 Gy, 50 Gy and the untreated situation are 16.3, 24.5, 30.6 and 5.6 days, respectively.

the latter tumors started at about 2 wk after treatment. The tumors exposed to doses of 10, 30 and 50 Gy had doubled their volumes present at the time of treatment (100%) after 18, 24 and 30 days, respectively. Since growth delays caused by the different treatments are dependent on the change in doubling time, growth delays of 1.98, 3.54 and 4.47 were calculated for the tumors treated with 10, 30 and 50 Gy, respectively (Table 3).

It is of interest to examine the combined effect of x-irradiation and hyperthermia on tumor growth. In Figure 4, growth curves are given for untreated tumors, tumors

TABLE 3
Effect of Radiotherapeutic and Hyperthermic Treatments on Tumor Growth of Rhabdomyosarcoma

Treatment	Growth delay (GD)	P value	Number
10 Gy	1.98 ± 0.09	$p < 0.004$	n = 8
30 Gy	3.54 ± 0.32	$p < 0.016$	n = 6
50 Gy	4.47 ± 0.38	$p < 0.016$	n = 6
15 min at 45°C	0.17 ± 0.04*	$p < 0.11$	n = 6
30 Gy + 15 min at 45°C	6.75 ± 0.04	$p < 0.07$	n = 4

Values are mean ± s.e.m.
P values obtained with Fisher's distribution free sign test (25).
* Data are from reference 15.

treated with hyperthermia during 15 min at 45°C, tumors treated with a dose of 30 Gy of ionizing radiation, and tumors treated with the combination. Hyperthermia alone had only a small effect on tumor growth as indicated by a GD of 0.17 (Table 3). Hyperthermia in combination with 30 Gy irradiation, however, resulted in significantly more inhibition of tumor growth than caused by 30 Gy alone. The radiosensitizing effect of hyperthermia can be quantified by using growth delay data. The GD of the combination of radiotherapy and hyperthermia, 6.75 as shown in Table 3, appeared to be larger than the summation of the GDs of the individual treatments (0.17 and 3.54). Consequently, a thermal enhancement ratio (6.75/3.71) of 1.8 may be calculated, quantifying the sensitizing effect of hyperthermia on the radiation treatment. A temporary increase in tumor volume, caused by edema, of about 30% was measured only on the first day after the combined treatment.

DISCUSSION

Tumors of rhabdomyosarcoma-bearing Wag/Rij rats were subjected to different doses of x-rays or to a combined

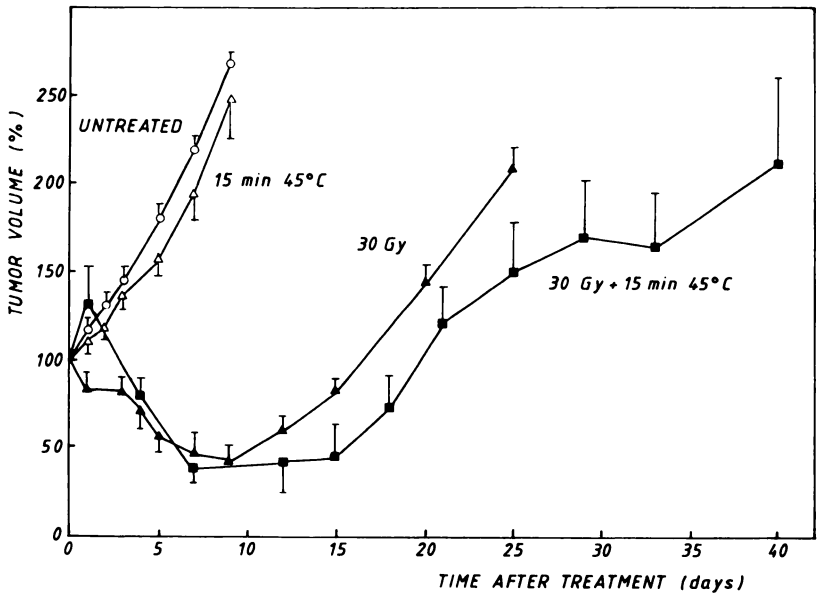

FIGURE 4. Growth curves of untreated rhabdomyosarcoma tumors (O, n = 9), tumors exposed to 30 Gy (▲, n = 6), tumors treated with hyperthermia for 15 min at 45°C (△, n = 6), and tumors treated with the combination (■, n = 4). The measured volumes (mean ± s.e.m.) are normalized to the volume at the moment of treatment. The mean doubling times for 30 Gy, hyperthermia only, its combination and the untreated situation are 24.5, 7.0, 39.1 and 5.6 days, respectively.

treatment of radiation and hyperthermia. Using PET, acute and indirect treatment effects were evaluated with ^{11}C-tyr and ^{18}FDG as metabolic tracers. For comparative reasons, radiation damage was also assessed with ^{14}C-tyr which is a probe to monitor amino acid uptake and incorporation into tumor proteins (*26*). The radiobiological effects on tumor growth were correlated with the metabolic data.

The ^{11}C-tyr data as measured by PET are in line with the effects on the ^{14}C-tyr uptake obtained in the corresponding dissection experiments. At 4 days after irradiation with 10 Gy, only a difference was observed between the ^{14}C-tyr data and the ^{11}C-tyr data. It is concluded that the ^{11}C-tyr data obtained by PET are a good reflection of tyrosine uptake and incorporation into proteins.

From in vitro studies, it is generally understood that immediate source acute effects on glycolysis and protein synthesis are not be expected from radiation doses in a therapeutic range up to 60 Gy (*3,4*). The absence of significant changes in ^{14}C-tyr, ^{11}C-tyr and ^{18}FDG uptake into rhabdomyosarcoma tumors measured directly after irradiation are in agreement with these conclusions.

In contrast to our observations, Kubota et al. (*9*) reported a rapid decreased uptake of L-[methyl-^{11}C]methionine into AH109A tumors at 6 hr after 20-Gy ^{60}Co irradiation. This discrepancy with our results may be explained by the difference in growth rate of the tumors. The AH109A tumor has a doubling time of 2 days, which is about half of the doubling time of the rhabdomyosarcoma tumor. Furthermore, in our study, the uptake of ^{11}C-tyr reflects protein synthesis. This was proved by the high incorporation percentages of ^{14}C-tyr into tumor proteins. Kubota et al. (*9*) investigated only the uptake of L-[methyl-^{11}C]methionine, and since this amino acid is involved in transmethylation it is unclear whether the rapid radiation-induced reduction of L-[methyl-^{11}C]methionine uptake reflects reduction of protein synthesis, transmethylation or amino acid transport.

After radiotherapy, unlike hyperthermia (*15*), only indirect metabolic effects on the tumor could be registered. Changes in tracer uptake in the tumor tissue, observed days after the irradiation, can be correlated with changes in tumor volume. The decline in the ^{11}C-tyr, ^{18}FDG and ^{14}C-tyr uptake, observed as indirect effects after the 30- and 50-Gy irradiations were accompanied with declining tumor volumes. Furthermore, in the 30-Gy experiments, restoration of tracer uptake to the value of the untreated situation, occurring between 4 and 12 days after treatment, was paralleled by a reverse in kinetics of tumor growth: from declining tumor volumes to progressive growth. In a rhabdomyosarcoma rat model closely related to our animal system, Jung et al. (*27*) observed that x-irradiation-induced decline in tumor volume was accompanied by a depopulation of the tumor cells per unit of volume, while after treatment recurrence of tumor growth was associated with repopulation. It is concluded that ^{11}C-tyr and ^{18}FDG are suitable indicators for depopulation and repopulation processes in tumors during and after radiotherapeutic treatment.

The fraction of ^{14}C-tyr incorporated into tumor proteins is not markedly affected after x-irradiation. This indicates that, although the tumor is depopulating or repopulating, the cellular protein synthesis is about constant. Notably, an unchanged fraction of ^{14}C-tyr incorporated into proteins was also found in necrotic parts of tumors 12 days after exposure to 10 Gy.

The extent of tumor growth delay is an indication for the effectivity of the treatment. In our investigation, the calculated GD correlated almost linearly with the dose of the irradiation.

Hyperthermia given as an adjuvant treatment to irradiation usually increases the effectivity of the irradiation (*23*). From the growth curves in Figure 4, it can be deduced that hyperthermia sensitizes the tumor tissue for radiation damage. This finding corresponds closely with the results of the study of Zywietz et al. (*28*) who found a thermal enhancement ratio of 1.7 in the rhabdomyosarcoma tumor after a combined treatment of 30-Gy x-irradiation and hyperthermia at 43°C for 60 min.

When comparing the growth data of the combined treatment with the corresponding rhabdomyosarcoma and ^{18}FDG uptake data, correlations, as observed in the single-dose irradiation experiments, were not found. This phenomenon is difficult to explain. Possibly, the time span of the PET studies (12 days) is too short to observe effects on metabolism. Furthermore, a pronounced edema of the tumor tissue was observed during a period of three days after the combined treatment. This may cause enhanced invasion of host cells, such as macrophages, as observed in radiotherapeutic studies of the rhabdomyosarcoma (*27*). These cells have high metabolic activities, and may largely contribute to the uptake value of ^{11}C-tyr and ^{18}FDG of the treated tumor tissue.

In conclusion, immediate radiation-induced effects on protein synthesis and glycolysis of tumor were absent. Indirect radiation effects on ^{11}C-tyr and ^{18}FDG uptake into tumor tissue correlated with radiation effects on tumor volume. Therefore, PET is suitable for investigations on tumor growth kinetics during and after a radiotherapeutic treatment, indicating tumor regression or recurrence of tumor growth. Since radiation-induced changes in ^{11}C-tyr uptake into rhabdomyosarcoma tissue are proportionate to changes in ^{18}FDG uptake, both tracers have an equivalent potential for assessing radiation damage to tumors by PET. In this study, radiotherapy in combination with hyperthermia did not affect tissue utilization of ^{11}C-tyr and ^{18}FDG. Therefore, PET data obtained during such combined treatments have to be interpreted with caution.

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