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Tumor Cell Spheroids as a Model for Evaluation of Metabolic Changes After Irradiation

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Tumor cell spheroids provide a good model to evaluate the relationship between tumor volume and the number of viable cells in the volume with the uptake of metabolic tracers before and after therapy. They represent the only in vitro model that allows the determination of the activity per unit volume, a parameter which is relevant for interpretation of PET studies. The purpose of this study was to evaluate this model with respect to the uptake of ^{14}C-FDG, ^{3}H-methionine and ^{3}H-thymidine with and without exposure to irradiation. **Methods:** Spheroids of the human adenocarcinoma cell line SW 707 were incubated in media containing ^{14}C-FDG, ^{3}H-methionine or ^{3}H-thymidine for 1 hr at 1, 4, 8, 24 and 48 hr after exposure to a single radiation dose of 6 Gy together with control spheroids. Tracer uptake after incubation was expressed in cpm/spheroid, cpm/1000 viable cells and cpm/0.01 mm^3. In addition, the proliferative capacity of control and irradiated spheroids was determined using the clonogenic assay. **Results:** Spheroid uptake of FDG decreased with time after irradiation, while the uptake per 1000 viable cells was increased significantly. The activity per unit volume remained unchanged in comparison to control spheroids. Methionine uptake per spheroid was unchanged after irradiation because of the high increase in uptake per 1000 viable cells. Uptake per unit volume also remained unchanged in comparison to controls. Thymidine uptake per 1000 viable cells did not change after irradiation but showed significant differences in uptake per spheroid and per unit volume compared to controls. The percentage of thymidine incorporated into the TCA-precipitable fraction containing DNA was 50% in controls and decreased to 12% at 24 hr after irradiation. The suppressed clonogenic capacity early after therapy recovered with the increase in thymidine uptake and with the increase in thymidine incorporation into DNA. **Conclusion:** The results show that the activity determined within a certain tumor volume is a balance between the increased tracer uptake by surviving cells after therapy and the lack of tracer uptake by dead cells, which still contribute to the tumor volume. Thus, the resulting unchanged activity per unit volume within the spheroid, as found for FDG and methionine, may not fully reflect therapy-induced metabolic changes in tumors.

Key Words: tumor cell spheroids; therapy monitoring; fluorodeoxyglucose; nucleotide and amino acid uptake; PET tumor tracers

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The use of radiolabeled 2-fluoro-2-deoxy-D-glucose (FDG), thymidine and amino acids in PET studies has been applied increasingly to assess tumor response to different therapy regimens through alteration in tumor metabolism. In addition to ^{18}F-FDG, other tracers, especially ^{11}C-L-methionine, have been used for PET studies since labeling of this amino acid with ^{11}C is relatively uncomplicated for routine production. While ^{18}F-FDG and ^{11}C-methionine uptakes reflect metabolic activity, the uptake of the nucleotide precursor, ^{11}C-thymidine, is considered a measure of proliferative activity.

Many clinical studies have demonstrated that a reduction of ^{18}F-FDG or ^{11}C-methionine uptake after therapy, as determined by PET, correlates to positive clinical results obtained by other methods. Experimental studies with transplanted tumors also demonstrated a rapid decrease in tumor uptake of ^{3}H-thymidine, ^{3}H-methionine and ^{18}F- or ^{14}C-FDG after irradiation, which preceded a change in tumor volume (*1–6*). However, the interpretation of the PET signal after therapy is still unclear. In some cases, tumor uptake remained at the pretreatment level or even increased after radiotherapy, despite a reduction in tumor mass. Although inflammatory tumor reactions with high metabolic activity in invading macrophages and granulocytes have been suggested to explain this phenomenon (*7*), in vitro studies with cultured human tumor cells also have shown a significant increase in uptake of metabolic tracers in the surviving tumor cells after irradiation or treatment with cytostatic drugs (*8–12*). Thus, it is important to investigate the relationship between tumor volume or number of viable cells and the uptake of FDG, thymidine and methionine, with and without exposure to irradiation, in a three-dimensional tumor model.

Multicellular tumor spheroids have several biologic characteristics in common with in vivo tumors. These three-dimensional cell aggregates represent models intermediate in complexity between two-dimensional monolayer cultures in vitro and transplanted tumors in vivo (*13–15*). Tumor spheroids with a diameter of 350 μm consist of approximately 12,000 cells, which allow penetration of tracers to the spheroid center within several minutes (*16,17*).

Similar to in vivo tumors, tumor spheroids are composed of cells with different metabolic activity and, consequently, with variable uptake of metabolic tracers. Since PET visualizes the mean uptake per unit volume in a tumor composed of cells of higher and lower metabolic activities as well as necrotic cells,

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the uptake of a tracer per spheroid corresponds to tracer uptake in a certain tumor volume in vivo. Therefore, multicellular spheroids are a suitable model for the interpretation of PET tracer uptake. Furthermore, this model also allows the determination of uptake only in viable cells and the calculation of the activity per unit volume.

The purpose of this study was to evaluate the spheroid model with respect to the uptake of ^{14}C-FDG, ^{3}H-methionine and ^{3}H-thymidine per spheroid and per 1000 viable cells obtained from disintegration of the spheroids, with and without exposure to irradiation. In addition, the changes in volume compared to the number of viable cells in control and irradiated spheroids also was investigated. From the uptake per spheroid and the spheroid volume, the mean uptake per unit volume can be calculated, which represents data that are obtained in PET studies. Thus, the evaluation of the uptake characteristics by surviving and dead cells, composing the spheroid, may lead to a better interpretation of PET studies.

MATERIALS AND METHODS

Spheroid Culture and Growth Studies

Cells from the human colon carcinoma cell line SW 707 were used for the culture of multicellular tumor cell spheroids using the liquid overlay technique. Briefly, cells (3×10^3 per well) were maintained in 96 multiwell plates coated with agarose and grown in RMPI 1640 media supplemented with 10% fetal bovine serum, 2 nmol L-glutamine, penicillin (100 IU/ml) and streptomycin (100 μg/ml). The concentrations of glucose, methionine and thymidine in the media were 1.0 mg/ml, 15 μg/ml and 1.85 μg/ml, respectively. All experiments were conducted in a humidified incubator containing 5% CO_2 at 37°C. The pH of the culture medium was kept at 7.4, and the media was renewed on Day 4 of the subculture. At this time, cells began to form and grow as spheroids that were kept in the media for 2 days before the start of the experiments.

Spheroid growth curves were established by replicate sampling (6×20 spheroids) per time point. The spheroid size was determined by measuring the maximum (a) and minimum (b) perpendicular diameters at 1, 4, 8, 24 and 48 hr after irradiation together with nonirradiated control spheroids using an inverted Olympus microscope. At the beginning of the experiments, spheroids were approximately 350 μm in diameter corresponding to a volume of 0.021 mm^3. The spheroid volume was calculated as $V = 4/3\pi ((a/2 + b/2)/2)^3$.

The number of viable cells per spheroid was determined at 1, 4, 8, 24 and 48 hr after irradiation together with control spheroids. To determine the number of viable cells, 6×20 spheroids at each time point were incubated for 5 min in 100 μl phosphate-buffered saline (PBS) containing 40 μl trypsin-ethylenediaminetetraacetic acid solution. To disintegrate the spheroid into single-cell suspension, they were separated with a pipette by moving them vigorously up and down. The trypsin reaction was stopped by adding 80 μl fetal calf serum. The viable cell number was assessed visually by trypan blue dye exclusion technique using a Neubauer hemocytometer chamber. In addition, spheroid volume and the number of viable cells were expressed as percent basal value (value for the control group at 1 hr after the beginning of the experiment).

Irradiation

Pilot studies have shown that Day 6 of the culture is the optimal time point for the initiation of the study. Before Day 6, spheroids are too small to reliably determine the number of viable cells per spheroid and the spheroid diameter and, therefore, to calculate spheroid volume.

Irradiation of spheroids on Day 6 of the culture with 2 and 4 Gy led to a small difference in viable cell numbers of irradiated spheroids in comparison to control spheroids. A 10-Gy irradiation was followed by a complete dissociation within 48 hr. Irradiation at 6 Gy, however, allowed follow-up of spheroid growth after irradiation and showed a significant reduction of viable cells. Therefore, spheroids in all experiments were exposed to a single dose of 6 Gy irradiation on Day 6 of the culture using the linear accelerator of a clinical radiation therapy unit (Mevatron; Siemens, Erlangen, Germany). The dose rate was 4.7 Gy/min. Control spheroids were sham irradiated at the same time.

Radiotracer Uptake Studies

Uptake studies were performed with 2-fluoro-2-deoxy D-^{14}C-glucose (specific activity 10.8 GBq/mmol), L-[methyl-^{3}H] methionine (3.15 TBq/mmol) or methyl-^{3}H-thymidine (1.7 TBq/mmol). All radioactive materials were obtained from Amersham (Braunschweig, Germany) and were diluted in RPMI 1640 media to obtain a final concentration of the incubation media of 37 kBq/ml for ^{14}C-FDG, and 370 kBq/ml for both ^{3}H-methionine and ^{3}H-thymidine.

The uptake experiments were initiated by choosing 5×20 spheroids from the same culture as used for establishing growth curves for each time point and each group visually using a microscope. They were collected into one well of a 24-well plate filled with 1 ml incubation media. Control and irradiated spheroids were incubated for 1 hr at 1, 4, 8, 24 and 48 hr after irradiation. Uptake of the radioactive substance was stopped by adding ice-cold culture media. Spheroids were transferred to wells with fresh, cold media and washed three times with 1 ml ice-cold media instead of PBS to avoid any change in the concentration gradient between the spheroid and the media. Afterwards, the spheroids were transferred into another cup and dissolved in 0.5 ml of 0.3 N NaOH and 1% sodium lauryl sulfate overnight. The solution was diluted in 10 ml scintillation fluid, and uptake of radioactivity was measured by liquid scintillation counting. The uptake was expressed as cpm/spheroid or as cpm/1000 viable cells. From the uptake per spheroid and the spheroid volume, the activity per unit volume was calculated and expressed in cpm/0.01 mm^3. Tracer uptake also was expressed as a percent of the basal value (control group at 1 hr after the beginning of the experiment).

In addition, we determined the relative incorporation of ^{3}H-thymidine into the high molecular weight fraction. At 1, 4, 8, 24 and 48 hr after irradiation, 5×10 spheroids were incubated for 1 hr in cups in incubation media containing ^{3}H-thymidine, as mentioned above, together with control spheroids. Uptake was stopped by adding 1 ml ice-cold media. After removal of the media, spheroids were washed three times with ice-cold media. Spheroids were transferred with 50-μl media into cups and, then, 0.3 ml cold, 10% trichloroacetic acid (TCA) was added. After 10 min, cups were centrifuged for 5 min. Supernatant was discharged, and the pellet was washed twice for 5 min. Subsequently, the pellet (TCA-insoluble fraction) containing DNA was resuspended in 0.3 ml of 0.3 N NaOH and 1% lauryl sulfate to dissolve the pellet overnight and also diluted into 10 ml scintillation fluid. Hydrogen-3 activity was measured in a liquid scintillation counter to determine the relative distribution in percent of ^{3}H-thymidine in the TCA-insoluble fraction.

Clonogenic Assay

The proliferative capacity of irradiated and control spheroids was assessed at 4, 24 and 48 hr using a clonogenic assay after disintegration of the spheroids as described earlier.

The number of cells was determined by a hemocytometer. One hundred cells from control spheroids were seeded in wells containing 2 ml medium. Three hundred cells in 0.1 ml from irradiated spheroids were handled in the same manner. After 9 days of culture, colonies were counted using a microscope. Cells giving

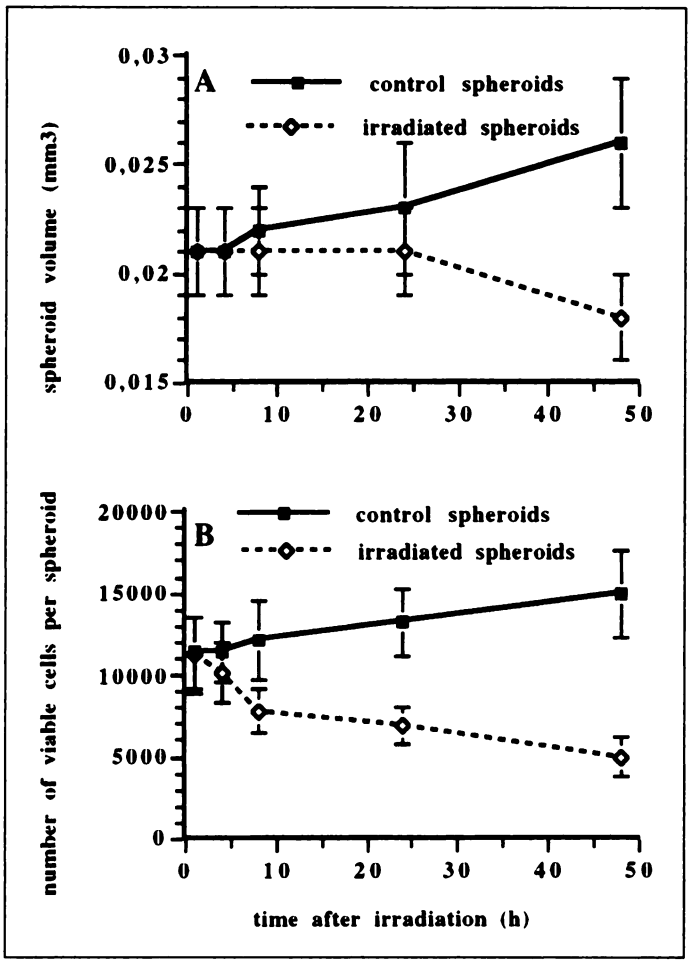

FIGURE 1. (A) Volume growth curve for control and irradiated spheroids up to 48 hr after irradiation (mean ± s.d., n = 60). (B) Cell growth curve for control and irradiated spheroids up to 48 hr after irradiation (mean ± s.d., n = 60).

rise to colonies larger than 50 cells were scored as proliferating. The plating efficiency of cells from untreated and treated spheroids was determined, and the proliferative capacity of irradiated spheroids was expressed as a percent of the control.

Histology and Macroautoradiography

Immediately after incubation, irradiated and control spheroids incubated with the tracer were washed twice in PBS, embedded in methylan and frozen to −26°C. Sections of 10 μm were cut in the cryostat, mounted on glass slides and placed in direct contact with radiographic film for 14 days to provide autoradiographic images. For histological examinations, spheroid sections were fixed and stained with hematoxylin-eosin.

Statistical Analysis

Tracer uptake was expressed as cpm/spheroid, cpm/1000 cells and cpm/0,01 mm^3. The values represent the mean of five determinations ± s.d. Statistical comparisons were based on unpaired Student's t-tests, and $p < 0.05$ was considered to be statistically significant. Linear regression analysis was used for assessing the correlation between spheroid volume and number of viable cells.

RESULTS

Spheroid Growth

The volume growth curves of SW 707 spheroids investigated over a time interval of 48 hr are shown in Figure 1A. At the time of irradiation with 6 Gy, the mean volume of spheroids was 0.021 ± 0.003 mm^3. It increased in control spheroids to 0.022 ± 0.003 mm^3 at 8 hr, to 0.023 ± 0.003 mm^3 at 24 hr, and to 0.026 ± 0.03 mm^3 at 48 hr. The volume of the irradiated spheroids did not change within 24 hr after irradiation. Between 24 and 48 hr, it decreased to 0.018 ± 0.002 mm^3, which is significantly lower than the corresponding value in the control group ($p < 0.01$).

Over the same time period, the number of viable cells per control spheroid obtained after spheroid disintegration showed an increase from 11352 ± 2156 to 14916 ± 2710 cells (Fig. 1B). A correlation between volume growth and the number of viable cells was found for control spheroids ($r = 0.96$, $p < 0.05$). After irradiation, the number of viable cells had decreased to 7800 ± 1326 cells within 8 hr. At 24 hr after irradiation, the number of viable cells was 6864 ± 1120, and showed a further decrease to 4979 ± 1210 at 48 hr. The difference in cell numbers between the two groups was significant at 8, 24 and 48 hr ($p < 0.01$). No correlation was found between spheroid volume and number of viable cells in irradiated spheroids.

While the volume of the irradiated spheroids did not change until 24 hr and decreased to 86% of basal value at 48 hr, the number of viable cells decreased to 69% at 8 hr and 44% at 48 hr. In control spheroids, the volume increased to 124% up to 48 hr and the number of viable cells increased up to 131%.

Histology and Macroautoradiography

Histological analysis of spheroid sections showed no sign of central necrosis in either group of spheroids. The periphery of the irradiated spheroid sections was not as smooth as that of control spheroids. In addition, the shape of the spheroids changed and did not allow an exact determination of the volume later than 48 hr after irradiation. Therefore, tracer uptake studies were performed up to 48 hr after irradiation.

All three metabolic tracers penetrated rapidly into the spheroids, showing a similar homogenous distribution pattern within several minutes after incubation. Therefore, the bioavailability for uptake appears to be the same for all the cells of the spheroid.

Uptake of Carbon-14-FDG

Carbon-14-FDG uptake within 48 hr after irradiation compared to the uptake in controls is shown in Figure 2A. Uptake per control spheroids showed a slight, but not significant, increase from 365 ± 36 cpm at 1 hr to 391 ± 35 cpm at 48 hr. Irradiated spheroids showed a significant decrease in uptake from 381 ± 35 cpm at 1 hr to 237 ± 30 cpm at 48 hr (65% of basal value, $p < 0.01$). The difference in uptake between the two groups was significant at 24 and 48 hr ($p < 0.01$).

FDG uptake per 1000 viable cells showed very different behavior (Fig. 2B). In viable cells of control spheroids, the uptake per 1000 cells showed only a slight decrease with time from 32 ± 6 cpm to 26 ± 5 cpm. Surviving cells from irradiated spheroids, however, demonstrated a significant increase in ^{14}C-FDG uptake from 33 ± 7 cpm at 1 hr after irradiation to 49 ± 9 cpm at 48 hr (149% of control value, $p < 0.05$). The difference between the two groups was significant at 24 and 48 hr.

The calculated values for the activity per unit volume expressed in cpm/0.01 mm^3 do not show significant differences between the two experimental groups over time (Fig. 2C). A nonsignificant decrease from 173 ± 13 cpm/0.01 mm^3 to 150 ± 12 cpm/0.01 mm^3 between 1 hr and 48 hr in the control group and 180 ± 24 cpm/0.01 mm^3 to 136 ± 23 cpm/0.01 mm^3 in the irradiated group was found.

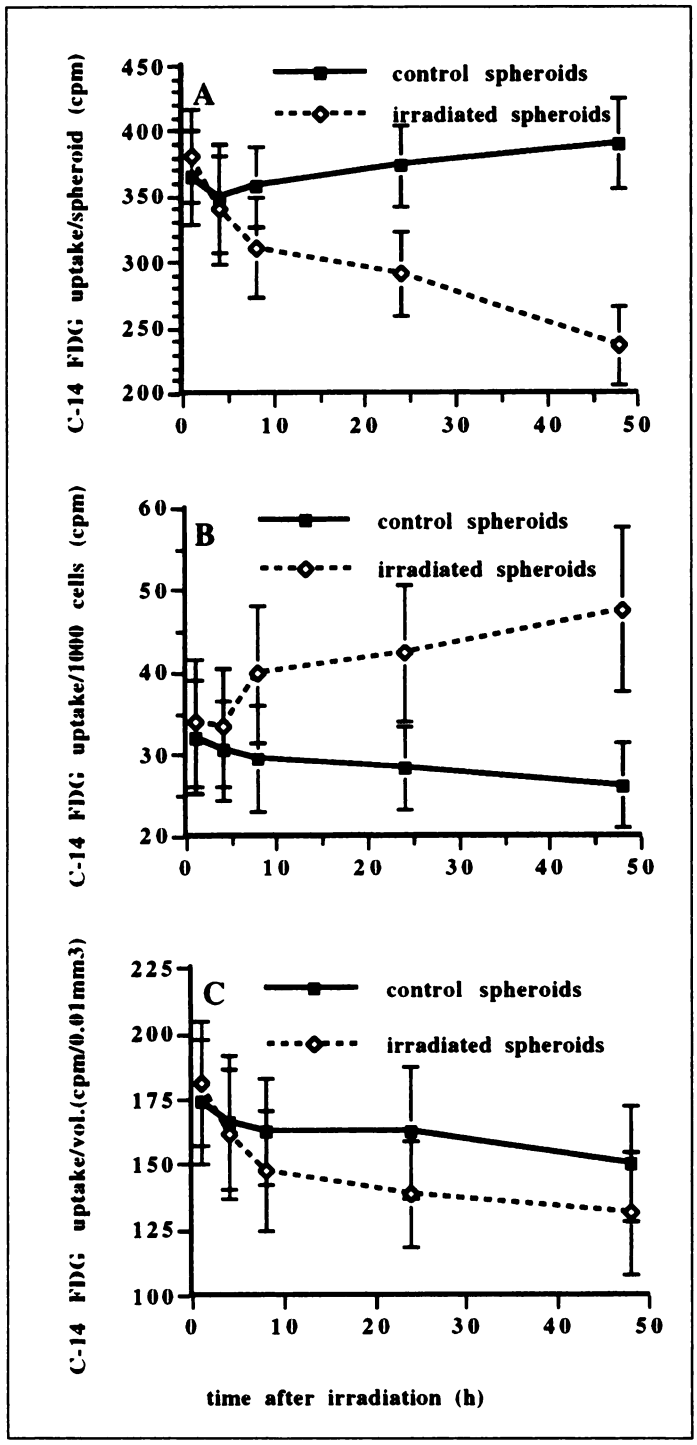

FIGURE 2. (A) Uptake of ^{14}C-FDG per spheroid (cpm) for control and irradiated spheroids up to 48 hr after irradiation (mean ± s.d., n = 5). (B) Uptake of ^{14}C-FDG per 10^3 viable cells for control and irradiated spheroids up to 48 hr after irradiation (mean ± s.d., n = 5). (C) Activity concentration (cpm/0,01 mm^3 of ^{14}C-FDG for control and irradiated spheroids up to 48 hr after irradiation (mean ± s.d., n = 5).

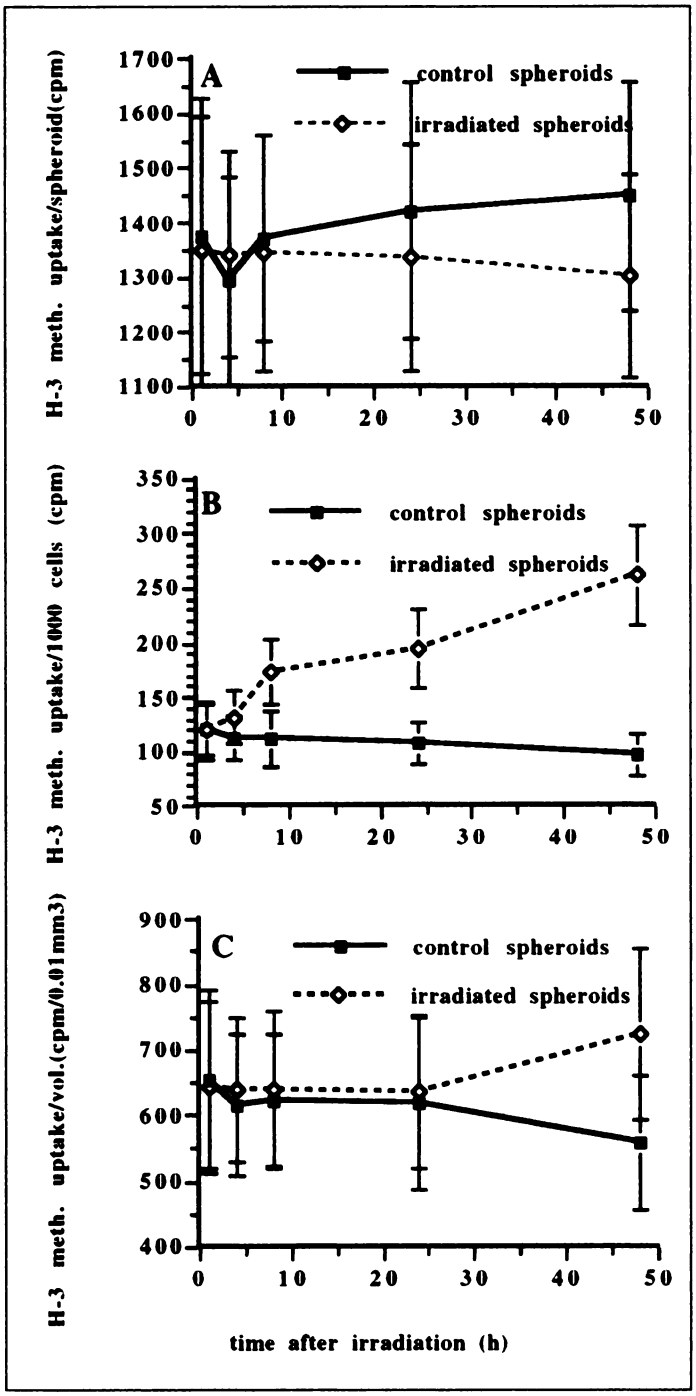

FIGURE 3. (A) Uptake of ^{3}H-methionine per spheroid (cpm) for control and irradiated spheroids up to 48 hr after irradiation (mean ± s.d., n = 5). (B) Uptake of ^{3}H-methionine per 10^3 viable cells for control and irradiated spheroids up to 48 hr after irradiation (mean ± s.d., n = 5). (C) Activity concentration (cpm/0.01 mm ^{3} of ^{3}H-methionine for control and irradiated spheroids up to 48 hr after irradiation (mean ± s.d., n = 5).

Uptake of Hydrogen-3-Methionine

The uptake of ^{3}H-methionine per spheroid (Fig. 3A) showed a slight increase for the controls from 1377 ± 252 cpm to 1450 ± 209 cpm. In irradiated spheroids, the uptake did not change between 1 hr and 48 hr after irradiation. The difference in uptake values between the two experimental groups was not significant at any time point.

The ^{3}H-methionine uptake per 1000 viable cells of control spheroids showed a slight decrease from 121 ± 21 cpm to 101 ± 19 cpm at 48 hr (Fig. 3B). Methionine uptake per 1000 viable cells in the irradiated group, however, showed a significant increase from 120 ± 23 cpm at 1 hr to 262 ± 44 cpm at 48 hr (217% of basal value, $p < 0.01$). At 8 hr after irradiation, the uptake per 1000 viable cells was already significantly different from that of the control group ($p < 0.01$). The calculated values for the activity per unit volume showed a slight, but not significant, decrease from 655 ± 135 cpm/0.01 mm^3 to 588 ± 103 cpm/0.01 mm^3 in controls and a slight increase from 643 ± 131 cpm/0.01 mm^3 to 715 ± 132 cpm/0.01 mm^3 in irradiated spheroids (Fig. 3C).

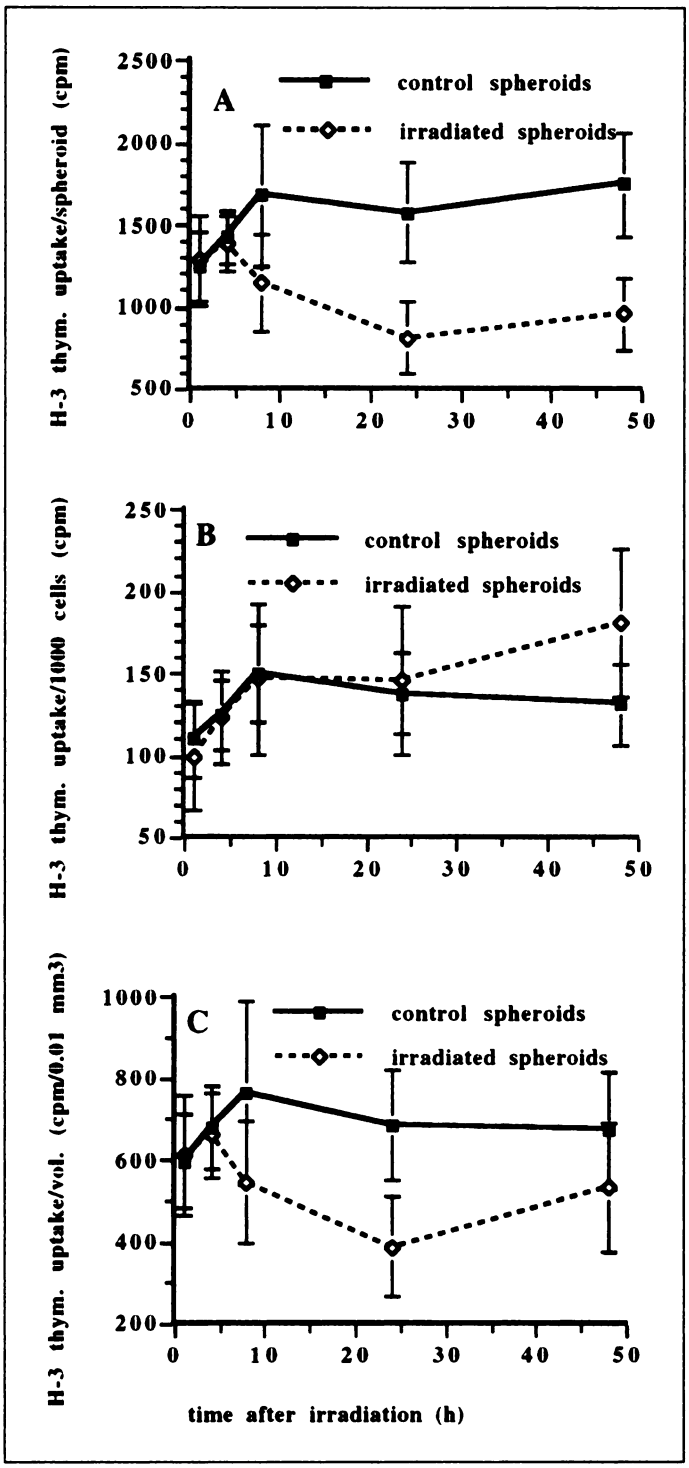

FIGURE 4. (A) Uptake of ^{3}H-thymidine per spheroid (cpm) for control and irradiated spheroids up to 48 hr after irradiation (mean ± s.d., n = 5). (B) Uptake of ^{3}H-thymidine per 10^3 viable cells for control and irradiated spheroids up to 48 hr after irradiation (mean ± s.d., n = 5). (C) Activity concentration (cpm/0.01 mm^3 of ^{3}H-thymidine for control and irradiated spheroids up to 48 hr after irradiation (mean ± s.d., n = 5).

Uptake of Hydrogen-3-Thymidine

Hydrogen-3 thymidine uptake per spheroid, per 1000 viable cells and per 0.01 mm^3 is shown in Figure 4. The uptake per spheroid in the control group showed an increase from 1251 ± 212 cpm at 1 hr to 1748 ± 277 cpm at 48 hr. The uptake per spheroid did not show any difference for both groups at 4 hr, but, thereafter, a decrease was observed for up to 8 hr for irradiated spheroids. The lowest uptake value of 815 ± 205 cpm was observed at 24 hr (65% of basal value, $p < 0.05$), followed by a slight, but not significant, increase up to 958 ± 211 at 48 hr (Fig. 4A). The differences to the control group were significant at 24 and 48 hr ($p < 0.01$).

No significant difference was found in uptake per 1000 cells between the two groups up to 24 hr. Until 8 hr after irradiation, both groups showed an increase in uptake. Between 24 and 48 hr, a slight decrease from 138 ± 23 cpm to 131 ± 28 cpm was found for the control group. In the irradiated group, an increase from 143 ± 35 cpm to 181 ± 38 cpm was observed (Fig. 4B).

A difference between the two experimental groups also was found for the activity per unit volume. At 8 hr, the uptake was highest in the control group, with an activity per unit volume of 765 ± 127 cpm/0.01 mm^3 followed by a slight decrease to 672 ± 134 cpm/0.01 mm^3 at 48 hr. A significant decrease in activity per unit volume in the irradiated spheroids was seen until 24 hr, to a level of 388 ± 103 cpm/0.01 mm^3 ($p < 0.05$). From 24 to 48 hr, an increase up to 532 ± 139 cpm/0.01 mm^3 was seen (Fig. 4C). The value was significantly different compared to the control at 24 hr ($p < 0.01$).

The relative distribution of ^{3}H thymidine into the TCA insoluble fraction in control and irradiated spheroids is shown in Table 1. In the control spheroids, approximately 50% of thymidine was incorporated into the TCA-insoluble fraction and did not show a change with time. The percentage of ^{3}H-thymidine in the acid insoluble fraction decreased significantly after irradiation to 11% at 8 hr, increasing to 22% at 48 hr. The difference to the control group was significant up to 48 hr ($p < 0.01$). These results indicate that, after irradiation, a much higher percentage of thymidine is not bound to DNA.

TABLE 1
Relative Distribution of Hydrogen-3-Thymidine in Trichloroacetic Acid-Insoluble Fraction in Control and Irradiated Spheroids up to 48 Hours*

Time after irradiation (hr)	Control spheroids	Irradiated spheroids
1	48 ± 5	25 ± 4
4	49 ± 3	17 ± 3
8	51 ± 2	11 ± 3
24	46 ± 3	16 ± 2
48	50 ± 3	22 ± 2

*Mean ± s.d., n = 5.

Clonogenic Assay

The results from the clonogenic assay showed a plating efficiency of 38% ± 6% for cells from the control spheroids at 4, 24 and 48 hr. The plating efficiency for the irradiated group was reduced to 1.7% ± 0.5% at 4 hr and 2.1% ± 0.6% at 24 hr after irradiation increasing to 8.3% ± 1.7% at 48 hr. From these values, a proliferative capacity of the irradiated cells of 4% was found at 4 and 24 hr after irradiation (control 100%). At 48 hr, an increase to 21% was determined.

DISCUSSION

Although PET has gained wide clinical acceptance in oncology, the interpretation of the registered PET signal, especially for evaluation of therapy response, is not fully established. Short-term assessment of therapy response generally involves the sequential registration of the accumulated activity in a certain tumor volume. This signal, however, may not always reflect the quantity of residual viable tumor cells. The signal may decrease due to cell death or increase due to enhanced uptake by surviving cells or due to infiltrating granulation tissue and macrophages. Thus far, studies of metabolic tracer uptake in tumor cells in vitro and solid tumors in vivo after irradiation

have reported diverging results. Most cell culture experiments have shown an increase in uptake of FDG, methionine and thymidine in surviving cells as a response to therapy. However, in in vivo studies tumor response to therapy is correlated with a decrease in tracer uptake due to the decrease in the number of viable cells as shown by histological findings (*5,18*).

To investigate the discrepancy between in vitro and in vivo results, a three-dimensional tumor cell spheroid model from SW 707 colon carcinoma cells was used to study the uptake of ^{14}C-FDG, ^{3}H-methionine and ^{3}H-thymidine with and without exposure to irradiation. This model allows the determination of the uptake per spheroid, per 1000 viable cells and per unit volume. The uptake per unit volume is the parameter relevant in clinical PET studies and can allow the comparison of the results from in vitro and in vivo studies. Therefore, spheroids seem to be an appropriate model to clarify, at least in part, the diverging results between in vitro and in vivo studies concerning the uptake of metabolic tracers in therapy monitoring.

In untreated spheroids, the data from this study showed a relationship between FDG and methionine uptake and the number of viable cells, as the uptake per 1000 cells did not significantly differ up to 48 hr. Similar results also were found for uptake per unit volume, indicating also a correlation between the number of viable cells and tumor volume. These results are in accordance with previously published results from in vitro and animal studies, which also suggest that FDG uptake is related to the number of viable cells and possibly related to tumor proliferation (*18–21*).

After irradiation, the uptake of FDG per spheroid decreased up to 48 hr, while the uptake per 1000 viable cells increased significantly in comparison to the control group. The uptake per unit volume did not show significant differences between the two spheroid groups. Thus, the lack of difference in uptake per unit volume may be a result of the uptake per spheroid and per viable cells, changing in opposite directions after irradiation. The decrease in tracer uptake per spheroid indicates a decrease in the number of tracer-accumulating cells in the spheroid, while the increase in tracer uptake per 1000 cells suggests an increase in tracer uptake by surviving cells. The fact that the activity per 1000 cells increases over time shows that the reported signal is a balance between the increased uptake by surviving cells after therapy and the increase in the number of dead cells that show a lack in tracer uptake but still contribute to the tumor volume. These results suggest that metabolic assessment after irradiation using tracer uptake per unit volume may be compromised by the opposing changes in the number of surviving cells and their metabolism.

An increase in uptake of FDG and methionine per 1000 viable cells after therapy was reported also by Higashi et al. (*12*). It was suggested that the increase in tracer uptake may be partially due to giant cell formation after irradiation. Giant cell formation, however, was not observed in this study.

Moreover, a study by Kubota et al. (*18*) reported that although giant cells were formed after irradiation of a transplanted tumor, the high tracer uptake was not influenced fundamentally by the giant cells but rather by membrane leakage. However, Haberkorn et al. (*9*) found that the increase in tracer uptake in cells treated with gemcitabine and incubated with ^{3}H-inulin was not caused by unspecific membrane alterations. Überall et al. (*22*) showed that treated cells exhibited normal levels of cytosolic Ca^{++} ions, which also provided evidence against extensive membrane disintegration or leakage as a consequence of therapy.

An explanation for the increased FDG uptake in cells after irradiation is the translocation of glucose transporters from the cytosol to the plasma membrane as a result of stress reaction. It also has been shown that physiologic stress, such as hypoxia, increased cellular uptake of FDG (*23*). The increase in glucose metabolism can be interpreted as a general feature of a stress reaction to a damaging agent (*24,25*).

From the three tracers investigated in this study, the uptake of methionine per 1000 cells showed the greatest increase after therapy (217% of basal value). However, there were no significant differences after therapy for uptake per spheroid and per unit volume compared to the control group. The increase in tracer uptake by surviving cells appeared to have completely compensated for the decrease in the number of viable cells. A comparative study between ^{14}C-methionine and ^{18}F-FDG uptake in a animal model showed a different distribution of the tracers in tumor tissue. While FDG accumulated in hypoxic and prenecrotic cells, as well as in macrophages and granulation tissue, ^{14}C-methionine mostly accumulated in viable tumor cells (*18*).

The accumulation of ^{11}C-methionine in the malignant tumor tissue is thought to be due to amino acid metabolism of cancer cells with increased transport and incorporation into the protein fraction. In addition to incorporation into protein ^{11}C-methionine also is incorporated into the lipid fraction and nucleic acid by transmethylation through S-adenosyl-l-methionine (*26*).

Studies by Ishiwata et al. (*27*). revealed that the uptake of methionine by tumor tissue reflected mainly amino acid transport rather than protein synthesis. After inhibition of protein synthesis in vivo a rapid accumulation of free methionine in a large intracellular pool was found in tumor and brain. In the liver a high uptake of ^{11}C-methionine mainly reflected phospholipid synthesis through the transmethylation process (*28*).

It is generally assumed that methionine is transported across the cell membrane mainly by the transport system L but also to a lesser extend by the systems A and ACS (*29*). Recent studies in our laboratory, inhibiting methionine uptake by the three different transport systems suggest that at physiological methionine concentration, this amino acid is taken up to a higher degree by unsaturable uptake systems or diffusion rather than by the known transport systems (*30*).

The uptake of radiolabeled thymidine generally is assumed to be an index of proliferative activity. This is confirmed by the increase in uptake of ^{3}H-thymidine in our control group. However, a significant reduction in uptake in irradiated spheroids was found at 24 hr, followed by a slight increase until 48 hr.

The uptake per 1000 cells did not show any significant difference between control and irradiated cells, despite the fact that the proliferation was tremendously inhibited as shown by the clonogenic assay. This is an indication that in surviving cells thymidine is found in different compartments within the cell compared to controls. A slight but nonsignificant increase is found between 24 and 48 hr. The activity concentration for this tracer is reduced in irradiated spheroids in comparison to controls with a significant difference at 24 hr after irradiation. Also, a slight increase is found between 24 and 48 hr. This increase is consistent with the increase in clonogenic capacity, obtained by the clonogenic assay. This is also in accordance with the result that incorporation of thymidine into DNA that was very low at 8 hr after irradiation increased up to 48 hr. Therefore, ^{3}H-thymidine incorporation into DNA seems to be related to the clonogenic capacity and is a measure of proliferation.

Shields at al. (*31,32*) have extensively investigated ^{11}C-thymidine and PET for noninvasively measuring DNA synthesis in tumors. They claim that, for interpretation of the

PET images, there must be detailed knowledge of thymidine metabolism and of the proportions of activity in the various chemical species, particularly the relative amount of activity incorporated into the DNA compared to that appearing in degradation products in the free cytosole pool.

In a recent study monitoring gene therapy with herpes simplex virus thymidine kinase in hepatoma cells, it was found that treatment with ganciclovir caused an increased thymidine uptake in the acid soluble cytoplasm fraction and a decrease in the acid insoluble nucleic acid fraction in TK expressing cells. Haberkorn at al. (*33*) suggest that there is an increase of activity of salvage pathway enzymes during repair of cell damage and this increase may be a reflection of cellular reaction to stress induced by therapy. To correctly interpret PET data using thymidine that represent the total activity in a certain tumor volume, additional information about thymidine metabolism and the amount of radioactivity in different cell compartments is needed.

CONCLUSION

Spheroids established from SW 707 human colon carcinoma cells are an appropriate tumor model for evaluating changes in uptake of metabolic tracers induced by irradiation. These changes can be related to changes in spheroid volume and in the number of viable cells. The model allows the determination of the tracer uptake per spheroid, per 1000 viable cells and per unit volume. The results obtained from these three parameters show that the uptake per spheroid, and per 1000 viable cells, can change in opposite directions. The reduced uptake per spheroid can be compensated by the increased uptake by surviving cells induced by therapy. The activity per unit volume is a balance between the increased tracer uptake by surviving cells after therapy and the lack of tracer uptake by dead cells, which still contribute to the tumor volume. Thus, the resulting activity per unit volume may not fully reflect therapy-induced metabolic changes in tumors. Although the decline in FDG, methionine and thymidine uptake reported for in vivo studies also may be due to additional factors, the data from this three-dimensional tumor model can give valuable information on the diverging results obtained between in vitro and in vivo studies using metabolic tracers for therapy monitoring.

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