In Vitro Assessment of 2-Fluoro-2-Deoxy-D-Glucose, L-Methionine and Thymidine as Agents to Monitor the Early Response of a Human Adenocarcinoma Cell Line to Radiotherapy

Kotaro Higashi,* Anaira C. Clavo and Richard L. Wahl

Division of Nuclear Medicine, Department of Internal Medicine, and Department of Radiology, University of Michigan Medical Center, Ann Arbor, Michigan

The tumor cell uptake of three tracers that can be labeled with isotopes suitable for PET imaging—FDG, L-methionine and thymidine—were examined in vitro in a human ovarian carcinoma cell line (HTB77IP3) at varying times following 30 Gy ^{60}Co irradiation and were compared to a nonirradiated control group. FDG, methionine and thymidine uptake per tissue culture well all *increased* following irradiation when compared to basal values, although to a much lower extent than the increases in uptake seen in a nonirradiated group. This increase in tracer uptake occurred despite a 6.25-fold decline in viable cell numbers. When examined per cell, FDG uptake per cell increased 9.77-fold, methionine 7.82-fold and thymidine 9.48-fold over basal levels from Day 0 to Day 12 following irradiation. Part of these increases may be due to giant cell formation and/or radiation repair processes that require energy, protein and DNA substrates. While the in vitro system differs from in vivo systems due to the absence of a blood supply in vitro, a lack of infiltrating leukocytes and other factors, our data suggest that early assessment of human adenocarcinoma response to radiotherapy by PET with these tracers may be complicated by this normal increase in tracer uptake postirradiation. Clearly, in this human cancer cell line, early radiation-induced cell death is not associated with an early decline in tumor cell uptake of FDG, methionine or thymidine.

J Nucl Med 1993; 34:773–779

Preclinical and clinical data support the feasibility of using positron-emitter labeled 2-fluoro-2-deoxy-D-glucose (FDG), thymidine and amino acids, such as L-methionine, to assess the response of cancers to radiation therapy (*1–7*), chemotherapy (*8,9*) and hyperthermia, particularly after treatments are concluded (*10*). For example, clinical studies using planar ^{18}FDG scanning have shown a substantial reduction in ^{18}FDG uptake from basal levels midway through a course of radiotherapy in patients who ultimately had a good response to radiation treatment of head and neck cancers (*3*). In animal models, Abe et al. (*4*) demonstrated that ^{18}FDG uptake decreased after irradiation of an animal tumor even when tumor volume was unchanged. Recently, Kubota et al. (*5*) demonstrated in vivo with an elegant quadruple-label technique in a rat hepatoma model that the in vivo tumor uptake of ^{3}H-thymidine and ^{14}C-methionine declined rapidly and were sensitive indicators of tumor response to irradiation, with declines in uptake preceding volumetric shrinkage and necrotic extension (*6*). The absolute signal strength and degree of change in tumor uptake seen with these tracers was not, however, as great as that seen with ^{18}FDG. Fluorine-18-FDG uptake in these tumors showed a wide range of change with a strong pretreatment signal and a steady decline in tumor uptake following radiation, which lagged behind changes in methionine and thymidine uptake. Fluorine-18-FDG uptake did parallel viable cell number decline (and the development of necrotic extension) preceding volumetric shrinkage (*5*).

The aforementioned studies, performed in animals and in patients, suggest that positron-emitting tracers may be useful in monitoring the radiation response of cancers. However, direct radiation effects (separate from those resulting from cell death) on glycolysis, protein synthesis or amino acid transport have been stated to be present only following high-dose irradiation (>100 Gy) in Yoshida ascites sarcoma or Ehrlich ascites carcinoma cells (*11,12*). In addition, Haberkorn recently described persistent uptake

Received Sept. 4, 1992; revision accepted Jan. 14, 1993.
For correspondence or reprints contact: Richard L. Wahl, MD, Division of Nuclear Medicine, University of Michigan Hospitals, B1G412, 1500 E. Medical Center Dr., Ann Arbor, MI 49109-0028.
*Current: Kanazawa Medical University, Department of Radiology, Kahoku-Gun, Ishikawa, Japan.

of FDG in colorectal cancers following high-dose irradiation (*13*). These changes have been attributed to "inflammation" induced by irradiation (*13*). Thus, it appeared important to clarify the relationship of FDG, methionine and thymidine uptake in tumor cells to cell viability following radiation treatment of a human adenocarcinoma cell line (HTB77IP3) free of contaminating inflammatory cells. This human adenocarcinoma would be relatively typical of the kinds of cancers that might be studied in patients with PET.

The ultimate goal of such studies is to develop an accurate noninvasive and early predictor of tumor radiosensitivity using the PET technique. In vitro studies of a pure population of the relevant cell population, tumor cells, may help define at what time following tumor irradiation PET examination for tumor response should be performed and allow us to better understand the metabolic processes associated with the radiation effect. For these reasons, we sequentially studied the uptake of FDG, methionine and thymidine in nonirradiated and irradiated human adenocarcinoma cells in vitro.

MATERIALS AND METHODS

Cell Culture

The human carcinoma of the ovary cell line, HTB77IP3 (IP3), was used. Cells (3×10^4/well) were inoculated into 24 multiwell plates and grown in RPMI 1640 media (Sigma, St. Louis, MO) supplemented with 7.5% calf serum, 2.5% fetal bovine serum, 2 m*M* L-glutamine, 100 IU/ml penicillin G and 100 μg/ml streptomycin. Final pH of the media was 7.4. Cells were incubated in a 5% CO_2 humidified incubator (Forma) at 37°C. The media was exchanged when the pH of the media fell under 7.4. Trypsin (0.05%), EDTA (0.53 m*M*), was used to dissociate the monolayers. Cell number in multiple replicate wells (n = 6) and cell viability were determined visually at regular intervals using the trypan blue exclusion method and an Olympus inverted microscope. These data were used to establish the cell growth curves.

Cobalt-60 Irradiation

Experimental tumor cells were exposed to a single dose of 30 Gy of ^{60}Co irradiation one day after subculture using a clinical radiation therapy device. The dose rate chosen was 0.672 Gy/min. The control group was sham-irradiated only.

Radiopharmaceutical Uptake Studies

At varying time points following inoculation into the multiwell plates, IP3 cells in replicate wells (n = 3) were preincubated in the RPMI media (selectamine RPMI media, Sigma) with a glucose concentration of 0.8 mg/ml, a L-methionine concentration of 15 μg/ml and a thymidine concentration of 1.85 μg/ml, and were then incubated in the RPMI media (1 ml) containing ^{3}H-FDG [5•6-^{3}H] (1 μCi [37 KBq], specific activity 40 Ci/mmol) [1.48 TBq/mmol], ^{3}H-L-methionine [methyl ^{3}H] (1 μCi [37 KBq], specific activity 85 Ci/mmol [3.145 TBq/mmol]) or ^{3}H-thymidine [methyl ^{3}H] (1 μCi [37 KBq], specific activity 45 Ci/mmol [1.665 TBq/mmol]) for 1 hr at 0, 1, 2, 3, 6, 8, 10 and 12 days after irradiation. Media glucose levels did not measurably change from basal levels during 1 hr incubation nor when several million cancer cells are incubated with the media for 4 hr. Thus, there would not be a change expected in FDG uptake due to changing glucose levels in the media. Studies performed using no glucose, methionine or thymidine media showed that ^{3}H-FDG, ^{3}H-methionine or ^{3}H-thymidine did not act as a tracer, rather radiopharmaceutical uptake per 10^5 viable cells declined as the cell number increased. This was not the case when the reaction was conducted at the 0.8 mg/ml glucose level, 15 μ/ml L-methionine level and 1.85 μ/ml thymidine level. Probably, radiopharmaceuticals with high specific activities account for this phenomenon. Glucose concentration (0.8 mg/ml) is equal to normal blood glucose levels for fasting humans. The L-methionine level (15 μ/ml) in the media was chosen to correspond to the upper part of the normal range of L-methionine levels reported in fasting normal males (*14*). Incorporation of radioisotopes was stopped by adding ice-cold Hank's Balanced Salt Solution (HBSS). The cells were then washed three times with HBSS and then dissolved in 0.5 ml of 0.3 normal NaOH plus 1% sodium lauryl sulfate. Cell extracts were diluted into 10 ml of scintillation fluid (Hionic Fluor, Packard, Meridien, CT), after which ^{3}H activity was measured with a liquid scintillation counter. Tracer uptake, expressed as percent dose per tissue culture well or percent dose per 10^5 viable cells, was determined. The percent dose per tissue culture well was felt to be most analogous to the percent uptake per "tumor."

We also studied the relative distribution of thymidine within the cancer cell in both the control and irradiated cell groups. IP3 cells in replicate wells (n = 3) were preincubated in the RPMI media mentioned above and incubated in the RPMI media containing ^{3}H-thymidine for 1 hr at 37°C at 0, 1, 2, 3, 6, 8 and 10 days after irradiation. Incorporation was stopped by adding ice-cold HBSS, washing the cells three times with HBSS and treating with 2 ml ice-cold 10% trichloroacetic acid (TCA) for 10 min and repeating TCA washes twice (5 min each) to extract DNA. Subsequently, the TCA-insoluble fraction (including DNA) was dissolved in 0.5 ml of 0.3 normal NaOH plus 1% lauryl sulfate and were diluted into 10 ml of scintillation fluid, after which ^{3}H activity was measured to estimate the relative distribution of ^{3}H-thymidine within the TCA-insoluble fraction (%TCA-insoluble fraction).

DNA Flow Cytometry Study

DNA flow cytometry was also performed to estimate synchronization of cell cycles in the irradiated group. IP3 cells (approximately 2×10^6 cells) cultured as above were trypsinized and washed with PBS at 0, 12, 24 and 48 hr postirradiation. These pellets were resuspended in 70% ethanol and kept at −20°C until staining. The samples were then treated with 0.5 ml RNase (54.5 μ/ml) for 1 hr at 4°C. Flow cytometry was performed with a Coulter Epics C Flow Cytometer. Histograms from the Epics C printout were used to estimate cell fractions in the G0/1, S and G2/M phases with commercial software (Dean's model, Coulter Inc., Hialeah, FL).

RESULTS

The cell growth curve is shown in Figure 1. Following subculture, the control cells progressed through a characteristic growth cycle, nearing the plateau phase at Day 12. Cell viability did not change following subculture in the control group and was always in excess of 91%. In the irradiated group, viable cell number decreased exponentially following irradiation and showed a 6.25-fold decline from Day 0 to Day 12. Cell viability also decreased gradually in the irradiated group, decreasing to 73% of the

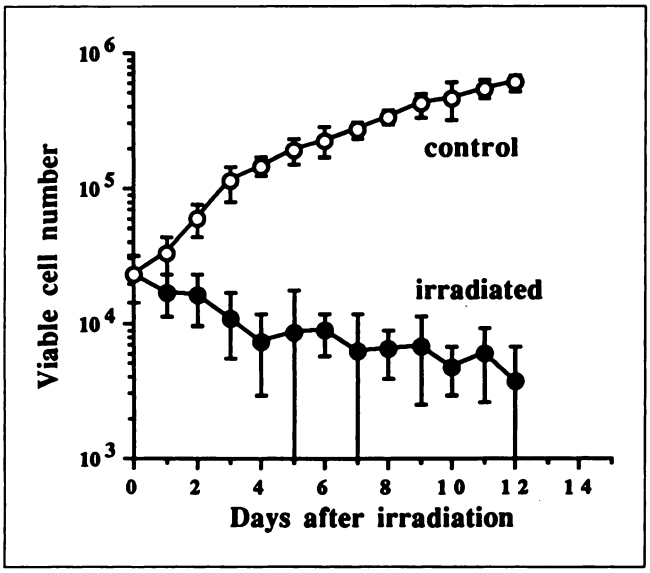

FIGURE 1. Cell growth curve. Following subculture, the control group (n = 6) progressed through a characteristic growth cycle. In the irradiated group (n = 6), viable cell number decreased exponentially following irradiation. Note that the y-axis is logarithmic. ○ = control group and ● = irradiated group.

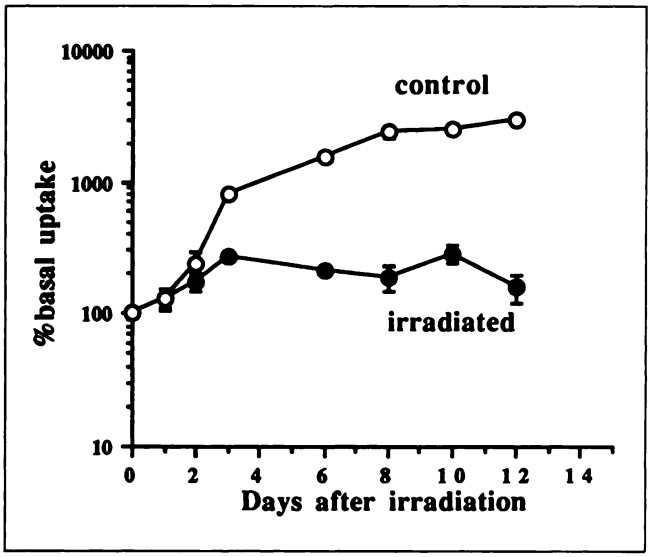

FIGURE 2. FDG uptake per tissue culture well increased slightly from the basal level in the irradiated group (n = 3), although to a much lower extent than that in the control group (n = 3). Measured uptake (mean ± s.d.) was normalized to the uptake at the time of irradiation. ○ = control group and ● = irradiated group.

residual visible cells at Day 12 following irradiation. Standard deviations on the irradiated samples were large relative to mean cell numbers at 5 days or longer postirradiation, due to the relatively low number of cells, and resultant low signal, present per well. The cell fractions in the G0/1, S and G2/M phases were determined by DNA flow cytometry in the irradiated group. Almost all cells (99%) entered the S phase at 12 hr following irradiation and 58% of the cells moved in the G2/M phase at 24 hr and were largely unchanged at 48 hr after irradiation. Thus, radiation-induced synchronization of the cell cycle was clearly apparent in the irradiated group. After 72 hr, however, a large amount of debris (dead cells) prevented the estimation of the cell fractions by DNA flow cytometry.

FDG uptake per tissue culture well was compared to basal FDG uptake at varying times postirradiation (Fig. 2). The percent dose of FDG uptake per tissue culture well increased over time in the control group in a manner paralleling the cell growth curve ($r = 0.974$, $p < 0.01$). There was a more modest increase in FDG uptake per tissue culture well in the irradiated group, but at all time points following irradiation, uptake in the irradiated group was greater than basal levels despite the fact that much fewer cells were present in the irradiated group at the later time points. FDG uptake was also examined per 10^5 viable cells. It was noted in this situation that there was more uptake of FDG relative to basal levels in the irradiated cells than in the control cells. This increase was substantial, a 9.77-fold increase from Day 0 to Day 12, which is relative to basal uptake (Fig. 3). Thus, a dramatic increase in FDG uptake per cell was seen following irradiation. Limited microscopic data showed that cell volume increased substantially in the residual irradiated cells that remained viable. These giant cells reached a volume roughly 11 times larger than control cells on Day 9 following irradiation. It is of interest that this increase in cell volume is similar in magnitude to the increase in FDG uptake per 10^5 viable cells. Thus, it is possible that this increase in FDG uptake per cell is largely related to an increase in cell volume (Fig. 4A,B).

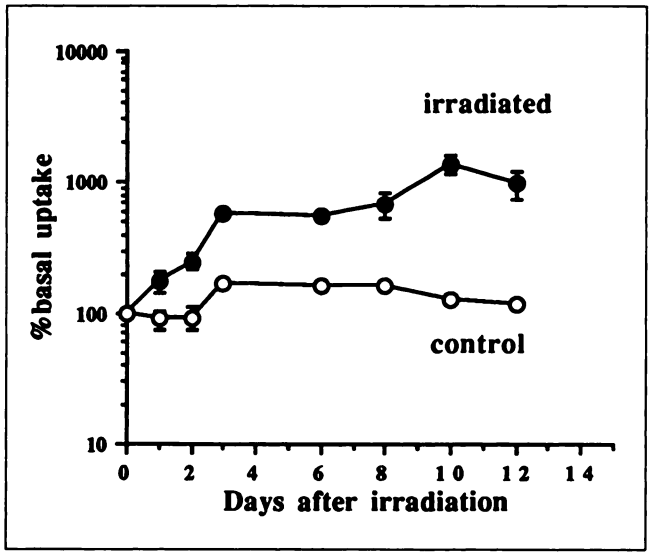

FIGURE 3. FDG per 10^5 viable cells increased markedly in the irradiated group (n = 3), with a 9.77-fold increase from Day 0 to Day 12, and increased to a much higher extent than that seen in the control group (n = 3). Measured uptake (mean ± s.d.) was normalized to the uptake at the time of irradiation. ○ = control group and ● = irradiated group.

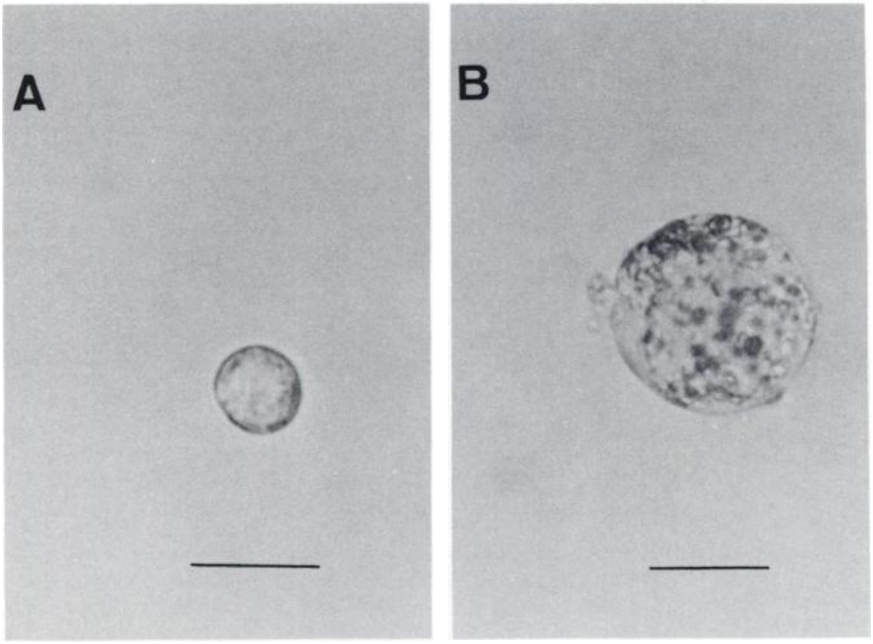

FIGURE 4. (A) Photomicrograph of control cell. (B) Residual irradiated giant cell at 9 days after 30 Gy irradiation. Volume has increased substantially in the remaining irradiated cells. Bar indicates 100 μm.

Methionine uptake per tissue culture well is shown in Figure 5. After irradiation, there was a modest increase in methionine uptake followed by a sharp decrease and another slight increase. Thus, there was a slight overall increase in methionine uptake in the irradiated cells, although it was much lower than in the control group. These data suggest that there is some cycling variability in methionine uptake following irradiation. Methionine uptake per 10^5 viable cells increased conspicuously in the irradiated group (Fig. 6), with a 782% increase from Day 0 through Day 12. This uptake per cell also tended to vary somewhat cyclically following irradiation and was much greater than the percent uptake seen in the control group. In fact, in the control group at the plateau phase, there appeared to be a slight decline in methionine uptake per cell relative to basal levels.

Thymidine uptake was examined as well. Thymidine uptake per tissue culture well increased in the control group from Day 0 to Day 12 (Fig. 7). There was cyclical variability in thymidine uptake in the irradiated group, with an increase in thymidine uptake seen at Day 3, although the increase was less marked at Day 8. There clearly was an increase in thymidine uptake per well after irradiation, although it was not as marked as the increase in FDG uptake.

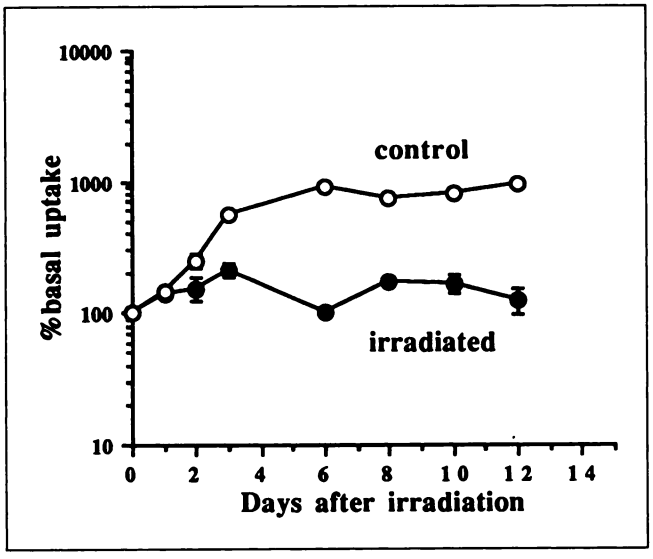

FIGURE 5. Methionine uptake per tissue culture well increased slightly from the basal level and varied in a cyclical pattern in the irradiated group (n = 3), although to a much lower extent than that in the control group (n = 3). Measured uptake (mean ± s.d.) was normalized to the uptake at the time of irradiation. ○ = control group. ● = irradiated group.

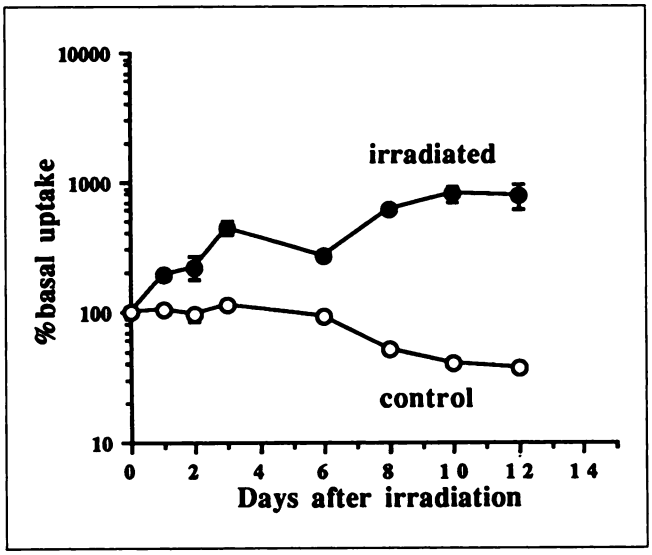

FIGURE 6. Methionine uptake per 10^5 viable cells increased markedly in the irradiated group (n = 3) with a 782% increase from Day 0 through Day 12 which tended to vary somewhat cyclically following irradiation and was much greater than the uptake seen in the control group (n = 3). Measured uptake (mean ± s.d.) was normalized to the uptake at the time of irradiation. ○ = control group. ● = irradiated group.

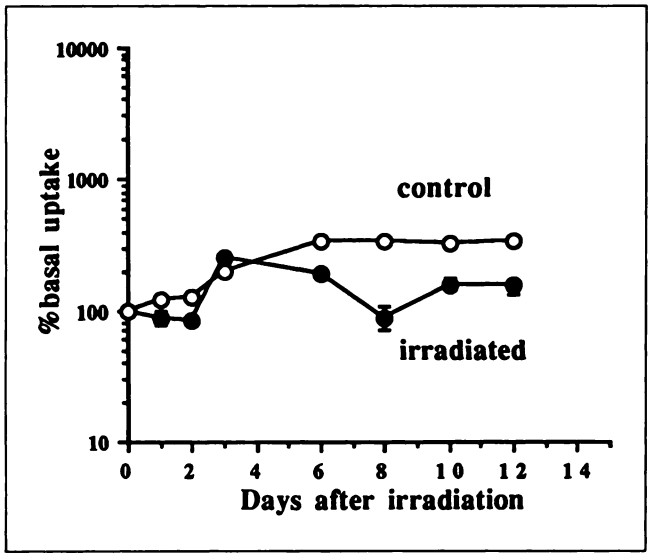

FIGURE 7. Thymidine uptake per tissue culture well increased in the control group (n = 3) from Day 0 to Day 12. There was cyclical variability in thymidine uptake in the irradiated group (n = 3). Measured uptake (mean ± s.d.) was normalized to the uptake at the time of irradiation. ○ = control group and ● = irradiated group.

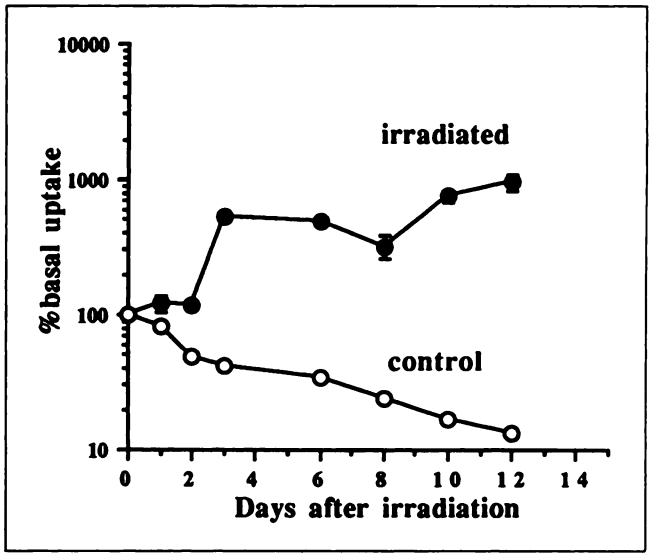

FIGURE 8. Thymidine uptake per 10^5 viable cells showed a progressive decline through the cell growth cycle in the control group (n = 3). By contrast, thymidine uptake per 10^5 viable cells in the irradiated group (n = 3) showed a 948% increase from Day 0 to Day 12 following irradiation. There also appeared to be cyclical variation of thymidine uptake in the irradiated group. Measured uptake (mean ± s.d.) was normalized to the uptake at the time of irradiation. ○ = control group and ● = irradiated group.

Thymidine uptake, when assessed per 10^5 viable cells, showed a progressive decline throughout the growth cycle of the cells in the control group (Fig. 8). This is consistent with the cells entering a slower phase of growth (and DNA synthesis) as they reach the plateau phase. By contrast, thymidine uptake per 10^5 viable cells in the irradiated group showed a 948% increase from Day 0 to Day 12 following irradiation. There also appeared to be cyclical variation of thymidine uptake, possibly due to radiation-induced synchronization of the cell cycle. We have also studied the relative distribution of thymidine within the cancer cells in both the control and the irradiated groups (Fig. 9). In the control cells, the %TCA-insoluble fraction for thymidine peaked at Day 1 and then decreased. In contrast, in the irradiated cells, the %TCA-insoluble fraction for thymidine increased gradually following irradiation, and at Day 10 virtually all of the thymidine was contained within the TCA-insoluble fraction (DNA). This indicates that thymidine accumulation in irradiated cells is mainly due to incorporation into DNA, while in control cells up to 50% of the thymidine is not in DNA. High thymidine incorporation into DNA in the irradiated cells is probably related to active repair of DNA damage from irradiation. These results indicate that irradiation can change the proportion of thymidine incorporation into DNA. When assessed per tissue culture well or per cell, there was *no evidence of a decline in tumor cell uptake of FDG, methionine or thymidine from basal levels in the first 12 days following irradiation.*

DISCUSSION

These data demonstrate that there was no decline in FDG, methionine or thymidine uptake per tissue culture well (i.e., per "tumor volume") following radiation therapy in an in vitro system using a human adenocarcinoma

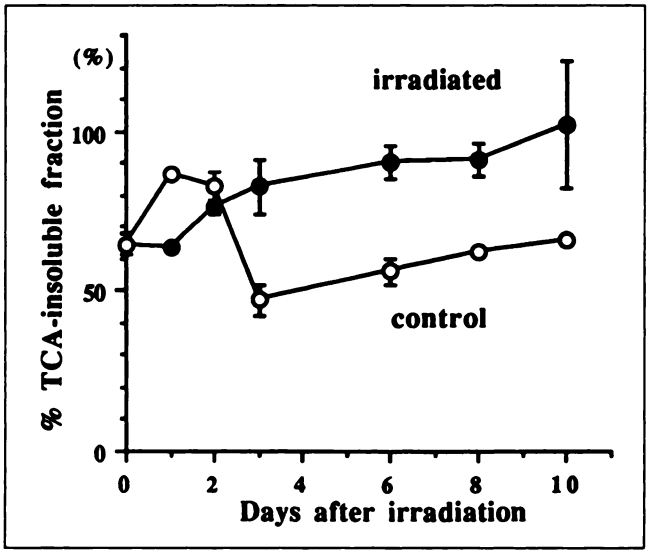

FIGURE 9. After irradiation, a significantly larger fraction of total ^{3}H-thymidine activity was incorporated into the TCA-insoluble fraction (DNA) versus the control group after Day 3. On Day 10, all ^{3}H-thymidine was contained within the TCA-insoluble fraction (DNA) in the irradiated cells. ○ = control group and ● = irradiated group.

of the ovary cell line which responds well to a 30-Gy radiation dose. In addition, since a decrease in viable cell number following irradiation was clearly apparent with cell counting, there was indeed a substantial increase in FDG, methionine and thymidine uptake per cancer cell following irradiation when the accumulated activity was normalized to the remaining number of cancer cells. Furthermore, a significantly larger fraction of the total ^{3}H-thymidine activity was incorporated into DNA versus the control group following irradiation, indicating thymidine utilization was increased in the irradiated cells. Part of the increase in tracer uptake per cancer cell may have been due to the fact that cell volume increases substantially following irradiation. This giant-cell formation following irradiation has previously been described and is likely due to continued protein, RNA and DNA synthesis and impaired cell division (*15,16*). It was also shown that the giant cells were metabolically active. Whitmore et al. (*15*) showed logarithmic increases in protein, RNA, DNA and cell volume over a 6-day period with strain L cells after 5000 roentgens irradiation. Sheet et al. (*16*) showed ^{14}C-thymidine uptake into cellular DNA of HeLa cells continued at essentially normal rates for 10 days after recovery from 1,400 roentgens of irradiation and then declined. The rate of incorporation of ^{14}C-glutamic acid reached a level greater than that in the control cells on a per cell basis. In an in vivo tumor system (neuroblastoma of mice) irradiated with 20 Gy in a single exposure, the tumor cells became considerably enlarged and pleomorphic as early as 3 days postirradiation, and the cellular changes were further exaggerated 6 days after irradiation (*15*). For example, this phenomenon could explain increased cellular FDG uptake and it is most consistent with the fact that FDG uptake increased the most per cell following irradiation. A similar explanation could hold for methionine uptake per cell. Giant-cell formation due to a lack of cell division coupled with increased DNA synthesis could also explain thymidine uptake increasing per cell. The 9.48-fold increase in thymidine uptake seen between Day 0 and Day 12 may also be due to repair of radiation damage in tumor cells that might require increased thymidine utilization. Thymidine and methionine uptake per cell varied in a cyclical pattern following irradiation. A DNA flow cytometry study showed that radiation-induced synchronization of the cell cycle was clearly apparent at 12 hr postirradiation. Part of the cyclical pattern for thymidine and methionine uptake may have been due to the synchronization of the cell cycle following irradiation.

Thus, our studies were somewhat surprising in that none of these tracers, which have been proposed as potential agents to monitor tumor response to radiation therapy, were sensitive at detecting early changes in viable cell number or in detecting cell death following cellular irradiation. Most surprising was that there was actually an *increase* in FDG, methionine and thymidine uptake, although particularly FDG, following such treatment per cell (where the increase is most dramatic) and per tissue culture well. Again, the tissue culture well is most analogous to the whole tumor in this in vitro system.

These in vitro findings in a human adenocarcinoma cell line are seemingly at variance with in vivo findings in systems examined by Kubota (radiosensitive rodent hepatoma, irradiation with 5, 10, 15 or 20 Gy in a single exposure) (*5,6*), tumors examined by Abe (radiosensitive and radioresistant rodent breast cancers, irradiation with 10, 20, or 40 Gy in a single exposure) (*4*) and head and neck squamous-cell carcinomas in patients examined by planar scintigraphy midway through the patients' course of radiation treatment by Minn (examined after irradiation with 30 Gy, daily fractions of 2 Gy) (*3*). Indeed, it may be that our in vitro model of a human adenocarcinoma is sufficiently different from the situation in vivo that extrapolation from in vitro to in vivo will not be valid. It is, however, possible that this adenocarcinoma is different (perhaps more radioresistant) than the other tumors examined.

It is perhaps more likely that the radiation-induced declines reported for in vivo systems in tumor FDG, methionine and thymidine accumulation may be due to factors other than direct radiation-induced cell death. For example, there might be radiation-induced vascular damage of tumors generated with resultant lower perfusion of tumors and less opportunity for these tracers to be accumulated in the tumors. In tumors (neuroblastoma of mice and Walker 256 carcinoma of rat) irradiated with more than 10 Gy in a single dose exposure, the intravascular volume decreased from Day 1 to Day 12 following irradiation, indicating severe vascular damage in these tumors (*17,18*). It is possible that some of the radiation effects reported with potential PET tracers for in vivo systems may be related to changes in tracer delivery, as opposed to direct radiation-induced decrements in cell number or metabolism. Alternatively, cell death following radiation may be more rapid in vivo than in vitro, perhaps due to the combined effects of direct tumor killing, radiation-induced changes in blood supply and radiation-induced edema and/or other host effector mechanisms, which possibly enhance the change in tracer signal beyond that seen in vitro. Of interest, however, is that Haberkorn and colleagues have reported substantial persistence of FDG signal in colon cancers following irradiation and have suggested that this is due to "inflammatory reactions" caused by radiation injury (*13*). Our data show that tumor cells can, at least transiently, increase their uptake of several PET tracers following irradiation without a need to explain the phenomenon as due to increased uptake into infiltrating inflammatory cells. Clearly, additional in vivo studies in this and other tumor systems are necessary to better assess and define the clinical relevance of this somewhat surprising in vitro phenomenon.

In the control cells, there are substantial differences in cellular uptake of thymidine and methionine between the

exponential and plateau phase cells. For both of these tracers, there is relatively less uptake per cell at plateau phase than during exponential phase. By contrast, FDG uptake per cell changes only minimally between the exponential and plateau phases. FDG uptake per tissue culture well, however, increased over time in a manner paralleling the cell growth curve ($r = 0.974$, $p < 0.01$). These in vitro differences in tracer uptake suggest that in this adenocarcinoma cell line, FDG measures a substantially different parameter (viable cell number) than thymidine or methionine and that thymidine and methionine uptake would underrepresent cell number if the cells are in the plateau phase in vivo and imaging is performed with PET.

In summary, our in vitro studies demonstrate that despite substantial reductions in viable cell number, there are *increases* in tumor cell FDG, methionine and thymidine uptake, both in absolute terms and per cancer cell, in a human adenocarcinoma cell line in the first 12 days following irradiation. These changes are partly due to giant-cell formation following irradiation, but may also be due to increased tracer uptake due to accelerated repair of cells, which require these substrates. From our in vitro data, it is clear that reductions in FDG, methionine and thymidine uptake do not occur shortly posteffective irradiation in this adenocarcinoma cell line. Our data also suggest that these agents may not be good tracers for *early* monitoring of the direct response of cancer cells to radiation. While extrapolations to the in vivo setting must be done with caution, our data suggest that caution is in order when performing PET imaging in the period during or soon after irradiation of adenocarcinomas, since it is clear that if proper cellular substrates are available, then tumor cell uptake of these three tracers *do not* rapidly decline, rather they *increase* in this human adenocarcinoma system. Additional in vitro and in vivo studies in animals and man will be necessary to address more comprehensively the issues of how PET tracer uptake changes in adenocarcinomas soon after successful and unsuccessful radiation therapy and whether such early radiation-induced changes in tumor metabolism are useful predictors of ultimate tumor response to treatment.

ACKNOWLEDGMENTS

The authors thank Barbara Burton for excellent secretarial assistance; David Kuhl, MD, for helpful discussions; and Susan Fisher, Gayle Jackson, MS, Raya Brown, PhD, Renato del Rosario, PhD, Jeannette Roesner and Phillip Sherman for technical assistance and advice. Supported by grants CA 53172 and CA 52880 awarded by the N.C.I., P.H.S. and by grant PDT 400 awarded by the American Cancer Society.

REFERENCES

1. Iosilevsky G, Front D, Bettman L, et al. Uptake of gallium-67-citrate and [2-^{3}H]deoxyglucose in the tumor model following chemotherapy and radiotherapy. *J Nucl Med* 1985;26:278–282.
2. Ichiya Y, Kuwabara Y, Otska M, et al. Assessment of response to cancer therapy using fluorine-18-fluorodeoxyglucose and positron emission tomography. *J Nucl Med* 1991;32:1655–1660.
3. Minn H, Paul R, Ahonen A, et al. Evaluation of treatment response to radiotherapy in head and neck cancer with fluorine-18-fluorodeoxyglucose. *J Nucl Med* 1988;29:1521–1525.
4. Abe Y, Matsuzawa T, Fujiwara T, et al. Assessment of radiotherapeutic effects on experimental tumors using ^{18}F-2-fluoro-2-deoxy-D-glucose. *Eur J Nucl Med* 1986;12:325–328.
5. Kubota K, Ishiwata K, Kubota R, et al. Tracer feasibility for monitoring tumor radiotherapy: a quadruple tracer study with fluorodeoxyuridine, L-[methyl-^{14}C]methionine, [6-^{3}H]thymidine, and gallium-67. *J Nucl Med* 1991;32:2118–2123.
6. Kubota K, Matsuzawa T, Takahashi T, et al. Rapid and sensitive response of carbon-11-L-methionine tumor uptake to irradiation. *J Nucl Med* 1989;30:2012–2016.
7. Daemen BJG, Elsinga PH, Paans AMJ, et al. Radiation-induced inhibition of tumor growth as monitored by PET using L-[1-^{11}C]tyrosine and fluorine-18-fluorodeoxyglucose. *J Nucl Med* 1992;33:373–379.
8. Minn H, Kangas L, Kellokumpu-Lehtinen P, et al. Uptake of 2-fluoro-2-deoxy-D-[U-^{14}C]-glucose during chemotherapy in murine Lewis lung tumor. *Nucl Med Biol* 1992;19:55–63.
9. Wahl RL, Cody R, Zasadny K, et al. Active breast cancer chemohormonotherapy sequentially assessed by FDG PET: early metabolic decrements precede tumor shrinkage [Abstract]. *J Nucl Med* 1991;32:982.
10. Daemen BJG, Elsinga PH, Mooibroek J, et al. PET measurements of hyperthermia-induced suppression of protein synthesis in tumors in relation to effects of tumor growth. *J Nucl Med* 1991;32:1587–1592.
11. Gammarano P. Protein synthesis, glycolysis, and oxygen uptake in hepatoma cells irradiated in vitro. *Radiat Res* 1963;18:1–11.
12. Archer EG. Inactivation of amino acid transport systems in Ehrlich ascites carcinoma cells by cobalt-60 gamma radiation. *Radiat Res* 1968;35: 109–122.
13. Haberkorn U, Strauss LG, Dimitrakopoulou A, et al. PET studies of fluorodeoxyglucose metabolism in patients with recurrent colorectal tumors receiving radiotherapy. *J Nucl Med* 1991;32:1485–1490.
14. Stegink LD, Bell EF, Filer LJ, et al. Effects of equimolar doses of L-methionine, D-methionine and L-methionine-dl-sulfoxide on plasma and urinary amino acid levels in normal adult humans. *J Nutr* 1986;116: 1185–1192.
15. Whitmore GF, Till JE, Gwatkin RBL, et al. Increase of cellular constituents in X-irradiated mammalian cells. *Biochim Biophys Acta* 1958;30: 583–590.
16. Sheek M, DesArmier R, Sagik BP, et al. Biochemical changes during the formation and growth of giant cells from irradiated Hela cells. *Exp Cell Res* 1960;19:549–558.
17. Song CW, Sung JH, Clement JJ, et al. Vascular changes in neuroblastoma of mice following X-irradiation. *Cancer Res* 1974;34:2344–2350.
18. Song CW, Levitt SH. Vascular changes in Walker 256 carcinoma of rats following X-irradiation. *Radiology* 1971;100:397–407.