Effect of Hyperglycemia on In Vitro Tumor Uptake of Tritiated FDG, Thymidine, L-Methionine and L-Leucine

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We have previously demonstrated in vitro and in vivo that tumor uptake of FDG is markedly diminished by acute hyperglycemia. This in vitro study was designed to determine if tumor uptake of PET tracers (FDG, thymidine, L-methionine and L-leucine) is affected by acute or chronic hyperglycemia. **Methods:** Human ovarian adenocarcinoma (HTB 77 IP3) cells were grown in media containing 100 or 300 mg/dl of glucose. At 7, 20, 38, 51 and 72 days after initial culture, uptake of 3H-labeled FDG, thymidine, L-methionine and L-leucine into the cells was determined in the presence of 100 or 300 mg/dl of glucose. **Results:** With acute hyperglycemia (300 mg/dl of glucose), the percent decreases in uptake of FDG, thymidine, methionine and leucine were 76.7%, 22.4%, 7.4% and 11.1%, respectively, as compared to assay at 100 mg/dl of glucose (mean day 51 and day 72 data). Significant decreases were observed in FDG and thymidine uptake with acute hyperglycemia (p < 0.0005). When cells grown at 300 mg/dl of glucose for 51 and 72 days were assayed at 100 mg/dl of glucose, the mean percent decreases in uptake of these tracers were 10.4%, 7.8%, 8.0% and 16.8%, respectively, as compared to cells grown and assayed at 100 mg/dl of glucose. No significant decrease was observed in tumor uptake of these tracers, except for leucine (p < 0.05). **Conclusion:** These human adenocarcinoma cells do not significantly change FDG uptake with chronic hyperglycemia while acute hyperglycemia markedly reduces uptake of FDG and thymidine. Neither methionine nor leucine uptake is significantly affected by acute hyperglycemia. To optimally evaluate tumor biology by PET, the fasting state seems necessary for FDG and thymidine studies, while methionine or leucine appears more suitable for hyperglycemic patients.

**Key Words:** fluorodeoxyglucose; nucleotide and amino acid uptake; hyperglycemia; cancer cell line; PET tumor tracers

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Previous in vitro and in vivo studies have demonstrated the feasibility of using positron-emitter labeled 2-fluoro-2-deoxy-D-glucose (FDG) (1-10). Thymidine and amino acids such as L-methionine and L-leucine are used to detect malignant lesions, which allow accurate staging of cancers and monitor therapeutic effects. We have previously reported that tumor FDG uptake is markedly diminished by acute hyperglycemia in vitro and in vivo because of direct competition between FDG and D-glucose for tumor uptake (11,12). In human studies, FDG-PET images obtained in either the fasting state or the glucose-loaded state have demonstrated that tumor FDG uptake is decreased, and thus the PET image quality is impaired when plasma glucose levels are increased (13,14). These results suggest that patients should fast before FDG-PET studies and their plasma glucose concentration needs to be considered when assessing tumor glucose metabolism (15).

Since many patients are diabetic and some diabetic patients also have cancers, it is important to determine if chronic exposure of cancer cells to hyperglycemia may influence glucose metabolism. In addition, little is known about the effect of acute or chronic hyperglycemia on tumor uptake of non-

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**REFERENCES**


FDG-PET tracers of tumor protein and DNA synthesis. In this in vitro study, human ovarian adenocarcinoma (HTB 77 IP3) cells were grown in media with 300 mg/dl of glucose, and the tumor PET tracer uptake into these cells was compared with that into cells grown at 100 mg/dl of glucose.

MATERIALS AND METHODS

Cell Culture
The human ovarian adenocarcinoma (HTB 77 IP3) cell line was obtained from ATCC (Rockville, MD) and handled as previously described (16). D(+)-glucose (10% w/v), obtained from Sigma (St. Louis, MO), was added to glucose-free RPMI to obtain the desired final concentration (100 or 300 mg/dl). Cells were seeded at an initial density of 0.2-0.3 x 10^6 cells per 150 cm^2 area tissue culture flasks, fed with a complete change in media on the third and fifth days and used or subcultured on the sixth or seventh day. At confluency, cells were dissociated with 0.05% trypsin-0.02% EDTA and used in the experiments or otherwise subcultured. Viable cell number was assessed by the Trypan blue dye exclusion technique using an Olympus (Lake Success, NY) IMT-2 inverted microscope. Cell viability was typically 80%-90%. All experiments were conducted in a humidified incubator containing 5% CO_2 at 37°C.

We monitored the media glucose level during cell growth because cancer cells may consume considerable glucose in growth media if glucose is not resupplied regularly. Glucose in the media was estimated with Accu-Chek IIm and Chemstrip bG (Boehringer Mannheim, Indianapolis, IN). DNA flow cytometric assays were also performed to assess the proliferative rate of the cells and use them near plateau phase as previously described (17).

Radioisotopic Tracer Uptake Study
Radiolabeled tracers used were FDG (2-deoxy-2-fluoro-D-[5,6-^3H] glucose), thymidine ([methyl-^3H]), methionine ([methyl-^3H] and leucine ([4,5-^3H]-N)) obtained from American Radiolabeled Chemicals Inc. (St.Louis, MO). These tracers were diluted in RPMI media with 100 mg/dl of glucose to obtain a final concentration of 37 KBq (1 µCi/100 µl) in the solution used in tracer uptake experiments.

Tracer uptake was performed as previously described (1). Briefly, after dissociation 1 x 10^6 viable cells in RPMI media (1 ml) with 100 or 300 mg/dl of glucose were aliquoted into sterile tubes. Radioactive tracer (1 µCi) was added to the cells and incubation continued for 60 min at 37°C. Hank's Balanced Salt Solution (HBSS), ice-cold, was used to stop tracer incorporation, and cells were washed three times with the same buffer. Cells were lysed in 0.5 ml of 0.3 M NaOH and 1% sodium dodecyl sulfate (incubated 30 min at room temperature). Whole cell extracts were mixed with 10 ml of scintillation fluid and kept overnight in the dark at 4°C. Bound radioactivity was measured by beta counting the following day in a 1600 TR Packard liquid scintillation analyzer. Tracer uptake was expressed as counts per minute per 1 x 10^6 viable cells and represents the mean of four determinations ± S.D. Statistical comparisons were based on unpaired Student's t-tests and p < 0.05 was considered to be statistically significant.

Tracer uptake assay was performed at 7, 20, 38, 51 and 72 days after the initial culture (6-7 days after subculture). Tracer uptake into cells was determined at the same media glucose level as the growth media; that is, cells grown at 100 mg/dl of glucose were assayed at 100 mg/dl of glucose and those grown at 300 mg/dl of glucose were assayed at 300 mg/dl of glucose. In addition, to determine if tracer uptake is affected by media glucose level at assay, tracer uptake for cells grown in media containing either glucose level was measured in the presence of 100 or 300 mg/dl of glucose at 51 and 72 days after the initial culture.

RESULTS

Media Glucose Levels
Until the cells were grown to confluence after feeding, the media glucose levels of cells grown at 300 mg/dl of glucose gradually decreased to about 200 mg/dl, and those of cells grown at 100 mg/dl of glucose gradually declined to about 50 mg/dl.

Effect of Hyperglycemia on FDG Uptake
Figure 1A shows that FDG uptake into the cells grown and assayed at 300 mg/dl of glucose was significantly reduced, as compared to cells grown and assayed at 100 mg/dl of glucose. After Day 20, FDG uptake into the cells grown and assayed at 300 mg/dl of glucose remained decreased, whereas FDG uptake into the cells grown and assayed at 100 mg/dl of glucose gradually increased after it dropped at Day 20.

As expected from our previous studies (11,12), when assayed at 300 mg/dl of glucose, FDG uptake was significantly reduced (76.7%) as compared to 100 mg/dl of glucose (Table 1). Table 2 reveals the percent decrease in tracer uptake into cells grown at 300 mg/dl of glucose relative to 100 mg/dl of glucose when cells were assayed at 100 mg/dl of glucose. FDG uptake into cells grown at 300 mg/dl of glucose had slightly reduced uptake (10.4%) as compared to 100 mg/dl of glucose and there was a significant difference between them only at Day 51. These results indicate that tumor FDG uptake was most significantly influenced by the media glucose level at the time of assay, not the glucose level during tumor cell growth.

Effect of Hyperglycemia on Thymidine Uptake
Thymidine uptake into cells grown and assayed at 300 mg/dl of glucose gradually increased and a more remarkable increase in uptake was observed in cells grown and assayed at 100 mg/dl of glucose (Fig. 1B). Thymidine uptake declined at Day 20 may be explained by the lower percentage of cells in S-phase observed at Day 20 when cells were grown at 100 and 300 mg/dl of glucose (11.2% and 9.6%, respectively), as compared to other days (mean 20.6% and 19.8%, respectively) (Fig. 2). A good correlation was observed between S-phase fraction and thymidine uptake.

Thymidine uptake significantly decreased when cells were assayed at 300 mg/dl of glucose as compared to 100 mg/dl of glucose, although the mean percent decrease (22.4%) was not so large as that of FDG uptake (Table 1). Thymidine uptake into cells grown at 300 mg/dl of glucose slightly decreased (7.8%) as compared to cells grown at 100 mg/dl of glucose (see Table 2).

Effect of Hyperglycemia on Methionine and Leucine Uptake
Uptake of methionine and leucine into cells grown and assayed at 300 mg/dl of glucose was higher at Day 7 but was lower after Day 20, as compared to cells grown and assayed at 100 mg/dl of glucose (Fig. 1C,D). These differences were modest but significant at Days 7, 20, 38 and 51 for methionine uptake and at Days 38, 51 and 72 for leucine uptake.

Tables 1 and 2 show that uptake of methionine and leucine decreased slightly, but in general not significantly, when cells were grown or assayed at 300 mg/dl of glucose. A significant difference was observed only when leucine uptake was compared between cells grown at 100 and 300 mg/dl of glucose.
FIGURE 1. Tracer uptake into cells grown and assayed at 100 mg/dl (gray column) and 300 mg/dl (white column) of glucose at 7, 20, 38, 51 and 72 days after initial culture. (A) FDG, (B) thymidine, (C) methionine and (D) leucine. Data represents the mean ± s.d. of four individual samples per condition. *p < 0.001, #p < 0.05.

DISCUSSION

These in vitro studies demonstrate that chronic exposure of this human adenocarcinoma cell line to hyperglycemia (300 mg/dl of glucose) only slightly reduced tumor uptake of FDG and non-FDG-PET tracers, as compared to tracer uptake of cells grown at the 100 mg/dl glucose level. Acute hyperglycemia significantly decreased FDG and thymidine uptake, whereas uptake of methionine and leucine was slightly but not significantly decreased under acute hyperglycemia. On the other hand, thymidine uptake of cells grown and assayed at 100 mg/dl of glucose tended to increase. This is quite apparent in Figure 1B, where thymidine uptake rises as the cells have better adapted to the culture condition. With each successive subculture for a longer period, the component of cells with the ability to proliferate more rapidly can gradually predominate while more slowly proliferating cells are selected out (18). This rise in

<table>
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<th>Tracer</th>
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<th>Day 72</th>
<th>Mean</th>
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*P < 0.0005.

Cells were grown at 100 mg/dl of glucose for 51 or 72 days.

<table>
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*P < 0.05.

Cells were assayed at 100 mg/dl of glucose.
thymidine uptake may in part reflect greater proliferation in cell culture. In our other studies, S-phase fraction and thymidine uptake were well correlated (Fig. 2).

In vitro and in vivo studies by Wahl et al. (11,12) have demonstrated that FDG uptake into human cancer cells and cancers is inhibited by increasing media glucose levels because of direct competition between FDG and D-glucose for uptake and incorporation into cultured tumor cells and cancers. Our data shown in Table 1 are consistent with these results. Clinical studies also have demonstrated that tumor FDG uptake is considerably decreased in hyperglycemic patients (13,14). These facts strongly suggest the importance of the fasting state for tumor FDG-PET studies to optimize tumor targeting. Since between 37% and 60% of cancer patients demonstrate glucose intolerance when subjected to a standard glucose tolerance test (19), it is important to determine if chronic exposure of cancer cells to hyperglycemia results in an alteration of tumor metabolism (i.e., increased glycolytic metabolism). On the other hand, some cancer patients have a tendency toward hypoglycemia because of increasing glucose demand by tumor growth (19,20). This latter condition appears to resemble the growth media with 100 mg/dl of glucose in our study, in which the glucose level ranged between normoglycemia and hypoglycemia until cells were grown to confluence after feeding.

It has been reported that expression of glucose transporters in some tissues is regulated by glucose levels in cell cultures (21-24). According to Simmons et al. (23), 24-hr treatment of fetal lung and muscle with high concentrations of glucose decreased 2-deoxyglucose uptake and Glut-1 protein and mRNA levels, whereas culture in low glucose media for 24 hr increased 2-deoxyglucose uptake and Glut-1 protein and mRNA levels. Expression of Glut-1 transporters is known to be influenced by media glucose levels. In addition, competition between FDG and D-glucose for hexokinase system may also be important because both substances compete for the same transport and kinase system (26). The reason that FDG uptake into cells grown and assayed at 100 mg/dl of glucose dropped at Day 20 is not clear. It should also be realized that mild day-to-day variation in tumor cell glucose uptake might be due to minor unmeasured variation in the cellular milieu.

FIGURE 2. Correlation between percent of S-phase fraction and thymidine uptake obtained at day 20, 38, 51 and 72. Each day has two data points from cells grown and assayed at two different glucose concentrations (100 and 300 mg/dl of glucose). The correlation \( r = 0.691 \) was good \( (p = 0.0576) \).

Tumor thymidine uptake declined significantly, but to a lesser extent than FDG, during acute hyperglycemia (Table 1), suggesting that tumor uptake of thymidine may be reduced at acutely elevated serum glucose levels and thus tumors potentially could be more difficult to image under such condition. An in vivo study by Googame et al. (27) demonstrated a 60% reduction in tumor DNA content after 5 days intravenous administration of 25% dextrose in rats. They also revealed that the decrease in DNA specific activity correlated with histological evidence of an increase in tumor necrosis, suggesting a toxic effect of hyperglycemia on tumor metabolism in vivo. However, this theory is in contrast to our in vitro result (Fig. 1B), in which thymidine uptake into cells grown and assayed at 300 mg/dl of glucose was gradually increased after Day 38 and excellent cell viability was maintained. The possible reason for this phenomenon is that cultured cells may gradually become more proliferative with continuous subculture for a longer period (18). This reason can be also applied to cells grown and assayed at 100 mg/dl of glucose, in which thymidine uptake was more elevated (Fig. 1B). Considering these cells were actually grown in a condition between normoglycemia and hypoglycemia, this elevation of thymidine uptake may agree with in vitro data, revealing that thymidine and glucose uptake was increased in relation to probable overexpression of Glut-1 transporters at low glucose concentration (28). Also in the in vivo studies, tumor DNA synthesis was increased in rats during the fasting state (29-31). Acute, but not chronic, hyperglycemia will reduce thymidine uptake in these tumor cells in vitro.

In contrast, since tumor uptake of methionine and leucine was less affected by acute hyperglycemia (Table 1), these tracers may be more suitable for hyperglycemic patients than FDG or thymidine. However, methionine and leucine uptake into cells grown and assayed at 300 mg/dl of glucose tended to reduce slightly after Day 20 (Fig. 1C,D), which may be due to impaired tumor metabolism during prolonged exposure of cells to hyperglycemia (20,25). Nevertheless, acute changes in glucose levels in tissue culture do not appear to significantly impact on tumor cell uptake of methionine or leucine.

CONCLUSION

With chronic hyperglycemia, the human adenocarcinoma cell line used in our studies does not significantly change glucose metabolism. Since acute hyperglycemia significantly reduces tumor FDG uptake, tumors in diabetic patients would be expected to have impaired FDG uptake during hyperglycemia, much as is seen in postprandial nondiabetic patients. Thymidine uptake also declines significantly during acute hyperglycemia, whereas tumor uptake of methionine and leucine is less affected by acute hyperglycemia. Therefore, to optimally assess tumor biology with PET, our in vitro study suggests that the fasting state appears optimal for FDG and thymidine studies, while...
Intrathecal 5-[\textsuperscript{125}I]Iodo-2'-Deoxyuridine in a Rat Model of Leptomeningeal Metastases

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In this model, 50% of rats develop paralysis of hind limbs in 9.20 ± 0.02 days after tumor-cell implantation and were killed 1, 8, 24, and 48 hr later. Tissues and organs, including the spinal cord, were isolated and their radioactive content determined. The results were expressed as percent injected dose per gram of wet tissue. Histological sections of the spinal cord were also prepared and used for autoradiographic detection of DNA-incorporated [\textsuperscript{125}I]UDR.

Treatment with i.t. administered [\textsuperscript{125}I]UDR (500 \textmu Ci/rat) significantly (p = 0.005) prolonged the median time of paralysis to 11.2 ± 0.1, 12.3 ± 0.1 and 15.2 ± 0.4 days for the single-dose, five daily injections and continuous infusion groups, respectively. Radioactivity cleared rapidly from all tissues except the thyroid and tumor cells growing within the spinal cord. Autoradiography demonstrated that normal cells in the tumor-bearing spinal cord were void of radioactivity. Conclusion: The results suggest that a selective antitumor effect could be achieved in treating leptomeningeal metastases with i.t. administered [\textsuperscript{125}I]UDR.

Key Words: leptomeningeal metastases; intrathecal tumor; iodine-125-UDR; gliosarcoma

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L eptomeningeal metastases are a serious complication of cancer characterized by neurologic dysfunction at multiple levels of the neuraxis. This disease develops in 5%–8% of patients with solid tumors, in 5%–29% of patients with non-Hodgkin’s lymphoma and in 11%–70% of patients with leukemia (7,2). The prognosis of patients who develop leptomeningin-
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