Fluorodeoxyglucose Uptake In Vitro: Aspects of Method and Effects of Treatment with Gemcitabine

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Rat prostate adenocarcinoma cells were used to evaluate different incubation procedures for the measurement of fluorodeoxyglucose (FDG) uptake and to measure the effects on chemotherapy. **Methods:** The cells were incubated for 10 or 60 min in media with different glucose concentrations. Furthermore, the cells were treated for 4 hr with different doses of gemcitabine. FDG uptake was measured immediately and 4 hr after treatment. The FDG transport was determined with a zero-trans assay, as well as the messenger RNA (mRNA) content of the glucose transporter type 1 (GLUT1) and the hexokinase assay (HK). **Results:** A decrease in FDG uptake with increasing cell number after 60 min of incubation in all media was found. The shorter incubation time yielded more stable uptake data. The glucose content in the medium decreased with increasing cell number and incubation time, which showed that the glucose-to-FDG ratio is not constant in assays that use glucose-containing media. Treatment with gemcitabine resulted in an increase in FDG uptake with increasing dose and time after the end of therapy. Incubation experiments with ^{3}H-inulin revealed that the changes were not caused by unspecific membrane alterations. The affinity (K_m) of the transport system remained unchanged, whereas the maximum velocity (V_{max}) increased. However, the mRNA content for GLUT1 and HK was unchanged. **Conclusion:** With these data in mind, an uptake procedure was suggested in a glucose-free medium with an end concentration of 0.1 m*M* FDG or a zero-trans assay to determine V_{max} and K_m of the transport system. In FDG-PET studies on patients with tumors, these in vitro data may be helpful to monitor and optimize the therapeutic outcome by combining the chemotherapeutic agent with low doses of deoxyglucose.

Key Words: FDG; PET; in vitro; glucose transporter; hexokinase

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Fluorodeoxyglucose (FDG) has been found to be a useful tracer in PET oncology studies, but the biologic interpretation of the FDG-PET signal in malignant lesions remains unclear to date. This problem can be addressed by in vitro approaches that allow the measurement of FDG uptake under controlled conditions. Data from these experiments may be used to develop working hypotheses for the interpretation of patient data. However, in vitro studies may suffer from methodologic impurities or from differences between the in vitro and the in vivo situation. One constraint of these vitro methods may be the unphysiologic medium formulation. Another one is the lack of the intercellular space, which causes local differences in pressure, pH, and so forth. However, these studies are valuable tools for the detection of general cellular reactions during malignant transformation or stress events, e.g., as a consequence of the exposure of cells to damaging agents.

Several studies have been undertaken in untreated (*1*) and treated cells (*2–4*). These used different experimental procedures to measure FDG uptake, e.g., 60 min of incubation either in glucose-free (*3,4*) or in glucose-containing media (*1,2*). However, it remains unclear, whether these methods are comparable in their reliability and significance. One fact not commonly recognized is that the glucose-to-FDG ratio may be changed during the experiment. Because glucose and FDG compete for transport and phosphorylation, this may result in alterations in FDG uptake. Another problem may be the influence of the cell number on the radiotracer concentration. Therefore, the objective of this investigation was to evaluate different incubation procedures with respect to the stability of the glucose content and the dependence of the radiotracer uptake on the cell number.

Furthermore, two optimized procedures were selected to measure the tumor cell reaction to a damaging agent. For this reason, FDG uptake was determined after chemotherapy with gemcitabine (difluorodeoxycytidine) [dFdC], a deoxycytidine analog with a broad activity against a panel of tumors (*5*). In addition, these data were compared with the expression of glycolysis-associated genes to evaluate whether the effects

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observed are caused by changes at the transcriptional or at the post-translational level.

The drug dFdC is metabolized intracellularly by nucleoside kinases to the active diphosphate (dFdCDP) and triphosphate (dFdCTP) nucleosides. The cytotoxic action of dFdC appears to be due to inhibition of DNA synthesis by two actions of dFdCDP and dFdCTP. First, dFdCDP inhibits ribonucleotide reductase, which catalyzes the reactions that generate the deoxynucleoside triphosphates for DNA synthesis. Inhibition of this enzyme by dFdCDP causes a reduction in the concentrations of deoxynucleosides in general, especially in that of dCTP. Second, dFdCTP competes with dCTP for incorporation into DNA. The reduction in the intracellular concentration of dCTP potentiates the incorporation of dFdCTP into DNA. After incorporation of dFdC into DNA, one additional nucleotide is added to the growing DNA strands and proofreading enzymes are unable to remove dFdC nucleotides from this position. Thereafter, a complete inhibition in further DNA synthesis occurs (masked chain termination).

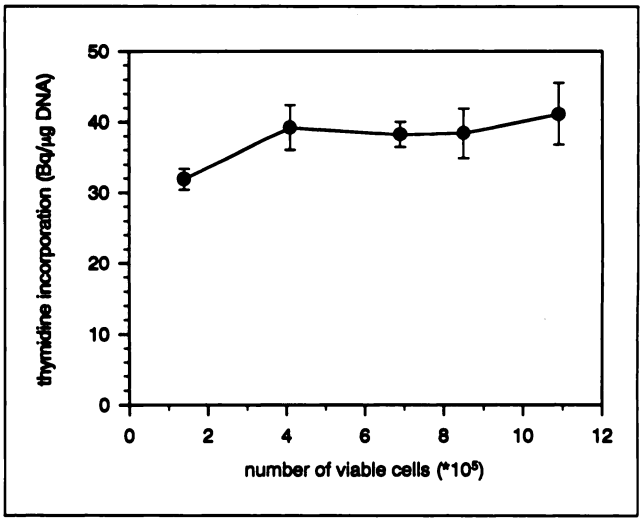

FIGURE 1. Thymidine incorporation into DNA versus viable cell number after 2-hr incubation with 2-^{14}C-thymidine. Mean values and s.d. (n = 3).

MATERIALS AND METHODS

Cell Culture

Rat prostate adenocarcinoma cells (Dunning R3327 rat prostate adenocarcinoma, subline AT1) were used for all experiments. The cells were maintained in culture flasks in RPMI-1640 medium (Gibco BRL, Eggenstein, FRG) supplemented with 292 mg/liter of glutamine, 100,000 IE/liter of penicillin, 100 mg/liter of streptomycin and 10% fetal calf serum at 37°C in an atmosphere of 95% air and 5% CO_2. Each of the following experiments was done in triplicate.

FDG Uptake In Different Media

The cells were trypsinized, and 10, 30, 50, 70 and 90 × 10^4 cells were seeded in six-well plates. Two days later, the uptake experiments were performed in the following different uptake media: glucose-free RPMI-1640 supplemented with glutamine and penicillin/streptomycin; glucose-free RPMI-1640 with glutamine, penicillin, streptomycin and 0.8 g/liter of glucose; or RPMI-1640 as provided by the manufacturer (glucose concentration 2 g/liter) with glutamine, penicillin, streptomycin and 10% fetal calf serum. After 30 min preincubation, 37 KBq (1 µCi) ^{14}C-FDG (Amersham, Braunschweig, FRG; specific activity 10.8 GBq/mmole; radioactive concentration 7.4 MBq/ml; radiochemical purity 99.3%) was added per milliliter of medium, and the cells were incubated for 10 or 60 min.

After this incubation period, the medium was removed, and the cells were washed twice with ice-cold phosphate-buffered saline (PBS). The lysis was done on ice with 200 µl of ice-cold 0.6 *M* perchloric acid and a cell scraper. Thereafter, the lysates were neutralized with 150 µl of 1 *M* KOH and 200 µl of 0.5 *M* Tris HCl (pH 7).

For scintillation counting, 300 µl of the lysates were mixed with 10 ml of Pico-Fluor-15 (Canberra Packard, Meriden, CT) and counted with a scintillation counter (LSC TRICARB 2500TR, Canberra Packard). The measured radioactivity was standardized to the viable cell number as determined by a Coulter counter (Coulter Electronics, Dunstable, UK) and the trypan blue method (more than 94% viable cells).

Thymidine Incorporation Into DNA

Because the growth cycle may be affected by the concentration of the seeded cells, the thymidine incorporation was measured 2 days after seeding of 10, 30, 50, 70 and 90 × 10^4 cells in six-well plates. The cells were pulsed with 2 µCi of 2-^{14}C-thymidine (Medgenix Diagnostics, Ratingen, FRG) for 2 hr in 1 ml of medium. After removal of the medium, the cells were washed three times with ice-cold PBS. The lysis was performed with 1 ml of 0.5 *M* perchloric acid and a cell scraper. After 30 min on ice, the lysate was vortexed and rotated at 1500 g for 5 min at 0°C. The supernatant was removed, and the pellets were washed with 1 ml of 0.5 *M* perchloric acid and rotated again for 5 min at 0°C. The pellet was resuspended in 1 *M* NaOH at 37°C. Aliquots were taken for scintillation counting and for DNA extraction. The DNA was extracted by the addition of sodium acetate and ethanol, and its content was measured photometrically at 260 nm. The radioactivity was then expressed as becquerels per microgram of DNA. The results are shown in Figure 1. No significant difference in thymidine incorporation was seen. This indicated that the cells were similar in proliferative activity.

Glucose Content In Uptake Media

The content of glucose in the uptake media was determined by the method described by Bergmeyer et al. (*6*) who used hexokinase (HK) and glucose-6-phosphate dehydrogenase. The amount of reduced nicotinamide adenine dinucleotide phosphate (NADPH) formed in this reaction is stochiometric with the amount of D-glucose. Briefly, 0.1 ml of sample was mixed with 1.9 ml of distilled water and 1 ml of a reaction buffer, which consisted of 0.3 *M* triethanolamine buffer (pH 7.6), 12 m*M* NADP, 150 m*M* adenosine-5′-triphosphate and 3 m*M* magnesium sulfate. The absorbances (A1) of these samples and the absorbances of a blank and standard suspension (0.5 g/liter) were determined 3 min later at 340 nm with an Ultrospec II photometer (Pharmacia, Freiburg, FRG). The standard suspension was used as an internal quality control in each experiment and revealed an error of the method below 3%. Thereafter, 20 µl of an enzyme solution (320 U of HK

and 160 U of glucose-6-phosphate dehydrogenase in 1.1 ml) were added and the absorbance was determined again 12 min later (A2). The absorbance differences (A2 − A1) were calculated for the blank and the samples, and the absorbance difference of the blank was subtracted from those of the samples. This yielded δA. The concentration (in grams per liter) was then calculated with the following formula:

$$c = ((V \times MW)/(6.3 \times d \times v \times 1000)) \times \delta A,$$

where c is the concentration, V is the final volume, MW is the molecular weight, d is the light path and v is the sample volume (6.3 is the absorption coefficient of NADPH at 340 nm).

Influence of Washing Procedure

To determine the influence of the number of washing steps on the uptake, the cells were incubated for 10 min in glucose-free RPMI-1640 or in RPMI-1640 with 0.8 g/liter of glucose after the addition of 37 KBq (1 μCi) of ^{14}C-FDG or 17.5 KBq (0.5 μCi) of ^{3}H-inulin (Amersham, specific activity 40 GBq/mmole, radiochemical purity 96.7%). Inulin is a slowly transported substance that can be used to measure the contribution of contamination caused by adhesion of radioactivity at the plasma membrane or at the bottom of the well or of unspecific membrane defects after incubation with damaging agents. After the incubation period, the cells were lysed without washing and after one, two or three washing steps with PBS for scintillation counting.

Determination of Maximum Velocity (V_{max}) and Affinity (K_m) of FDG Transport

A zero-trans uptake assay (*7–11*) was performed with different end concentrations of FDG (0, 0.05, 0.2, 0.6, 1, 2.5 and 5 m*M*). The V_{max} and K_m of the FDG transport system were determined by nonlinear-regression analysis (*12*) of the velocity V versus the substrate concentration (S) with the Michaelis-Menten equation

$$V = V_{max} \cdot [S]/(K_m + [S]).$$

After 30 min of preincubation in glucose-free RPMI-1640 with glutamine, penicillin and streptomycin, the cells were incubated for 10 min in glucose-free RPMI-1640 with 37 KBq (1 μCi) of ^{14}C-FDG and cold FDG at the mentioned end concentrations.

Effects of Treatment with dFdC

The tumor cells were treated for 4 hr with dFdC (provided by Lilly, Bad Homburg, FRG) at concentrations of 10, 50 and 100 n*M*. The FDG uptake was determined immediately and 4 hr after the end of treatment at an end concentration of 0.1 m*M* FDG and 10 min of incubation time. The possible contribution of unspecific membrane alterations was evaluated by uptake experiments with ^{3}H-inulin. Moreover, the cell volume was determined in controls and treated cells.

Furthermore, V_{max} and K_m were measured in controls and in cells treated for 4 hr with 100 n*M* dFdC immediately and 4 hr after treatment.

RNA Extraction and Northern Gel Blot Analysis

Total cellular RNA was isolated in controls and treated cells with the acid-guanidinium-thiocyanate-phenol-chloroform method (*13*). The cells were lysed in a denaturing solution (solution D) with 4 *M* guanidinium thiocyanate, 25 m*M* sodium citrate (pH 7), 0.5% sarcosyl and 1% 2-mercaptoethanol. To the lysate in 1 ml of solution D, 0.1 ml of 2 *M* sodium acetate (pH 4), 1 ml of water-saturated phenol and 0.2 ml of chloroform-isoamyl alcohol (a mixture of 49:1) were added. The suspension was mixed and cooled on ice for 15 min. After centrifugation at 10,000 g for 20 min at 4°C, the aqueous phase was transferred into another tube, mixed with 1 ml of isopropanol and stored at −20°C for 1 hr. An additional centrifugation step at 10,000 g for 20 min was done. The pellet was dissolved in 0.3 ml of solution D and precipitated with one volume of isopropanol at −20°C. After centrifugation for 15 min at 15,000 rpm in an Eppendorf centrifuge, the RNA pellet was washed in 75% ethanol and dissolved in water. The concentration was determined photometrically at 260 nm.

Then 20-μg aliquots of RNA underwent electrophoresis in 1% agarose-formaldehyde gels, which were blotted with nylon membranes (Hybond N, Amersham) in 20 × SSC buffer (20 × SSC: 3*M* NaCl, 0.3 *M* Na_3 citrate · $2H_2O$ adjusted to a pH of 7 with HCL). The hybridization was performed with complementary DNA probes for the glucose transporter type 1 [GLUT1, obtained from M. Mueckler (*14*)], for HK [obtained from K. Arora (*15*)] and for beta-actin (*16*), according to the method described by Church and Gilbert (*17*). The membranes were hybridized overnight at 65°C in hybridization buffer (0.5 *M* sodium phosphate, pH 7.2, 7% sodium dodecyl sulfate (SDS) and 10 m*M* edetic acid (EDTA).

The probes were labeled with ^{32}P-dCTP (Amersham-Buchler) by use of a random primer labeling system (Gibco BRL). After hybridization and washing in 40 m*M* sodium phosphate at pH 7.2 and 1% SDS, autoradiography was done with Kodak X-OMAT TM AR films (Eastman Kodak, Rochester, NY) for 4 hr (beta-actin) or for 24 hr (GLUT1 or HK). The autoradiograms were then scanned with a Sharp JX600 scanner (Osaka, Japan) and analyzed by densitometry with purpose-designed software (Cirrus, Canto, Hamburg, FRG). Because the scanner is not linear at the upper limit of the gray values, care was taken to shorten the exposure time of the membranes to the film. The indicated times result in gray values within the linear range. Thereafter, the GLUT1 and HK signals were standardized to the beta-actin signal.

RESULTS

Figure 2 shows the influence of the viable cell number on the FDG uptake in the different uptake media. After 60 min of incubation, a decrease in FDG uptake with increasing cell number was seen in all media (Figs. 2A and B). No significant influence of the cell number on the FDG uptake was found after 10 min of incubation (Figs. 2C and D). An additional experiment with an end concentration of 0.1 m*M* FDG in glucose-free medium also showed no cell number-related changes (Fig. 2C). Furthermore, the Michaelis-Menten kinetic characteristics for FDG transport was determined 2 days after seeding of $2 \cdot 10^4$ or $7 \cdot 10^4$ cells and yielded no significant differences in the V_{max} and K_m values (Fig. 3).

The medium glucose content was determined in the 0.8-g/liter and 2-g/liter media after 10 min (30 min of preincubation and 10 min of uptake experiment) and 60 min (30 min of preincubation and 60 min of uptake experiment) incubation. A decrease to 78% was found (Figs. 4A and B), and a dependence of the glucose content on the viable cell number was seen with r = 0.96 (40 min) and r = 0.99 (90 min) in the 0.8-g/liter medium, and r = 0.92 (40 min) and r = 0.94 (90 min) in the 2-g/liter medium [all significant at the 1% level (*18*)].

The dependence of the medium's glucose content on the incubation time for two cell numbers (3.3×10^5 cells and

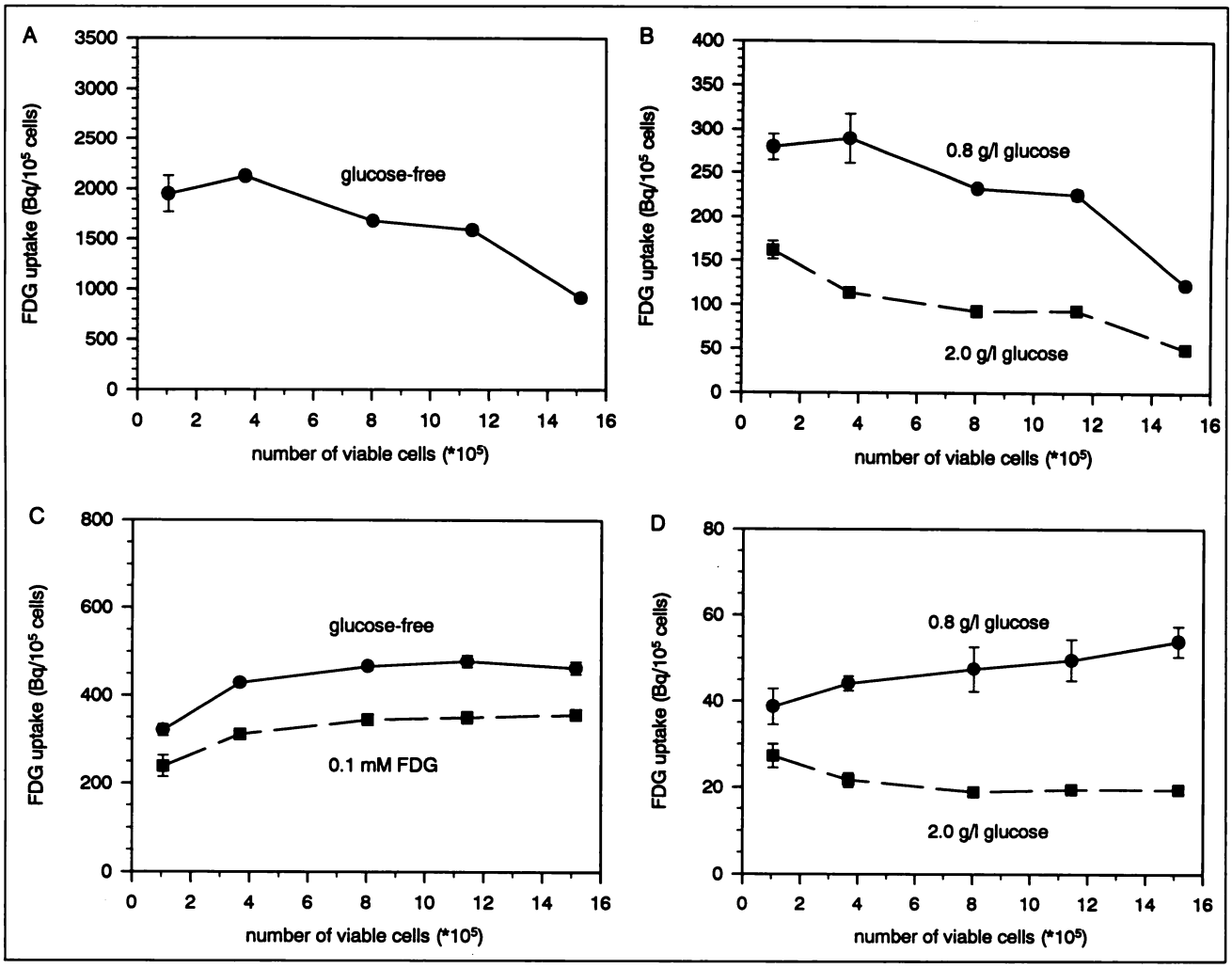

FIGURE 2. FDG uptake versus viable cell number in different uptake media after 60-min (A and B) or 10-min (C and D) incubation. Mean values and s.d. (n = 3).

1.4×10^6 cells) is shown in Figure 4C. After 5 min of incubation, a decrease was observed to 94% and 89%, respectively. The changes in glucose content were time-dependent with r = 0.89 for the lower cell number and r = 0.84 for the higher cell number (both significant at the 5% level). In addition, the influence of the washing procedure on the radioactivity measured was determined with FDG and inulin in glucose-free medium and medium that contained 0.8 g/liter glucose. After two washing steps with PBS, the data were found to be stable for both tracers (Fig. 5).

The effects of chemotherapy with 10, 50 and 100 n*M* dFdC are shown in Figure 6A. A dose- and time-dependent increase in FDG was found. To determine whether the increase in FDG uptake was caused by unspecific membrane alterations, the cells were incubated with ^{3}H-inulin, and no difference in inulin uptake was found (Fig. 6B). The cell volume was 1.159 to 1.338 fl in controls, 1.182 to 1.338 fl after 10 n*M*, 1.182 to 1.383 fl after 50 n*M* and 1.18 to 1.330 fl after 100 n*M* dFdC. The evaluation of the drug effects on FDG transport immediately and 4 hr after therapy revealed

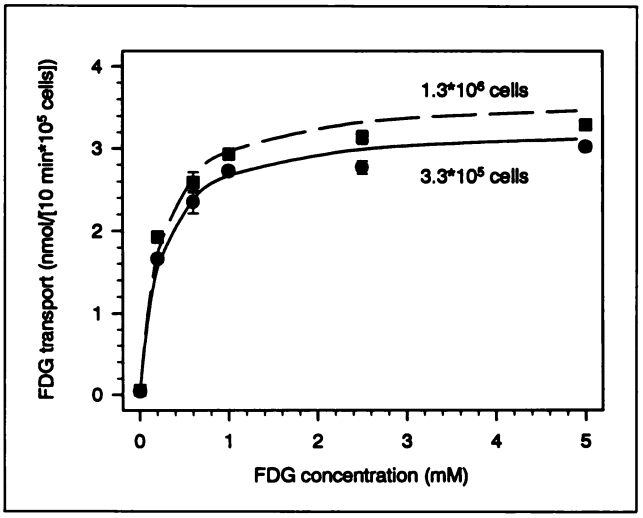

FIGURE 3. Michaelis-Menten kinetics of FDG transport for $3.3 \cdot 10^5$ and $1.3 \cdot 10^6$ cells. V_{max} was 3.26 ± 0.11 for the lower cell number (K_m 0.222 ± 0.029) and 3.61 ± 0.19 for the higher cell number (K_m 0.219 ± 0.012). Mean values and s.d. (n = 3).

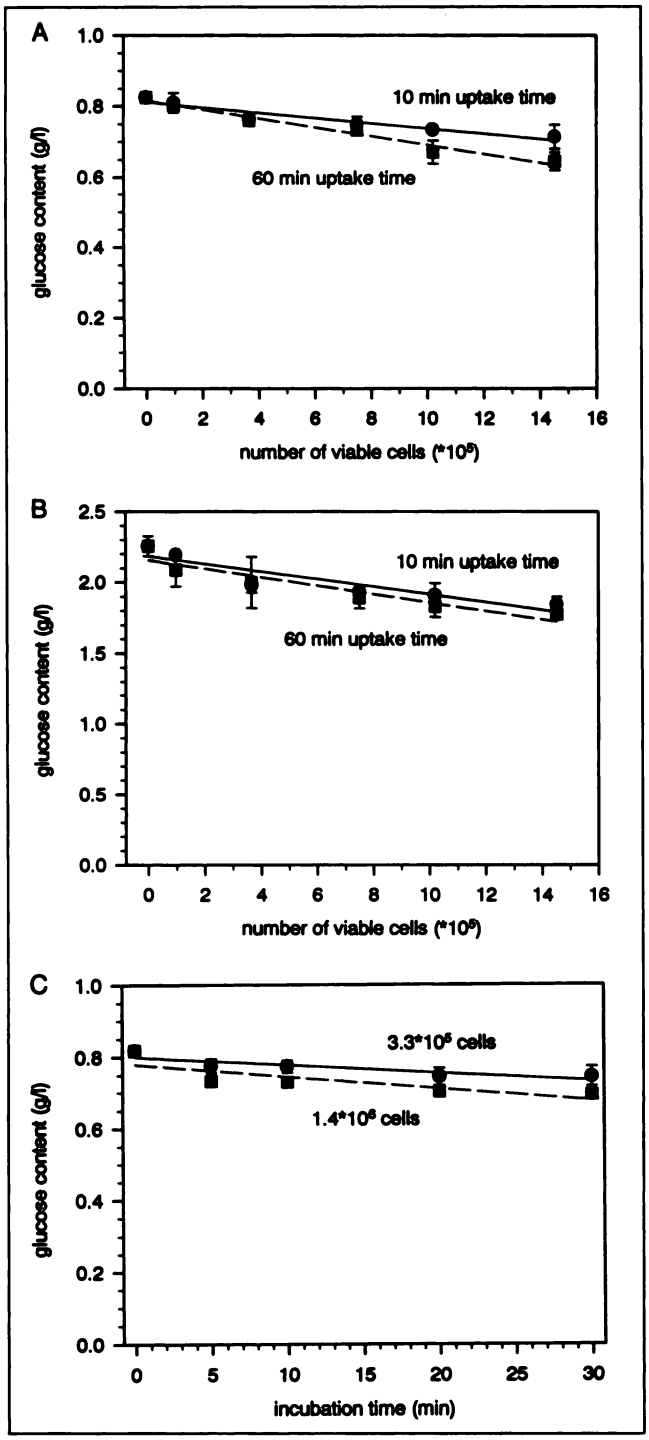

FIGURE 4. Medium glucose content versus viable cell number in the 0.8-g/liter (A) and the 2-g/liter (B) medium. (C) Dependence on the incubation time for $3.26 \cdot 10^5$ and $1.44 \cdot 10^6$ cells in the 0.8-g/liter medium. Mean values and s.d. (n = 3) and regression lines.

an increase in V_{max} and no significant change in K_m (Table 1 and Figs. 6C and D). In contrast, the analysis of the messenger RNA (mRNA) content for GLUT1 and HK showed no difference between controls and treated cells (Fig. 7). In addition, high-performance liquid chromatography was done as described (*4*). It showed that 88% ± 0.8% (n = 6) of the FDG was phosphorylated in controls

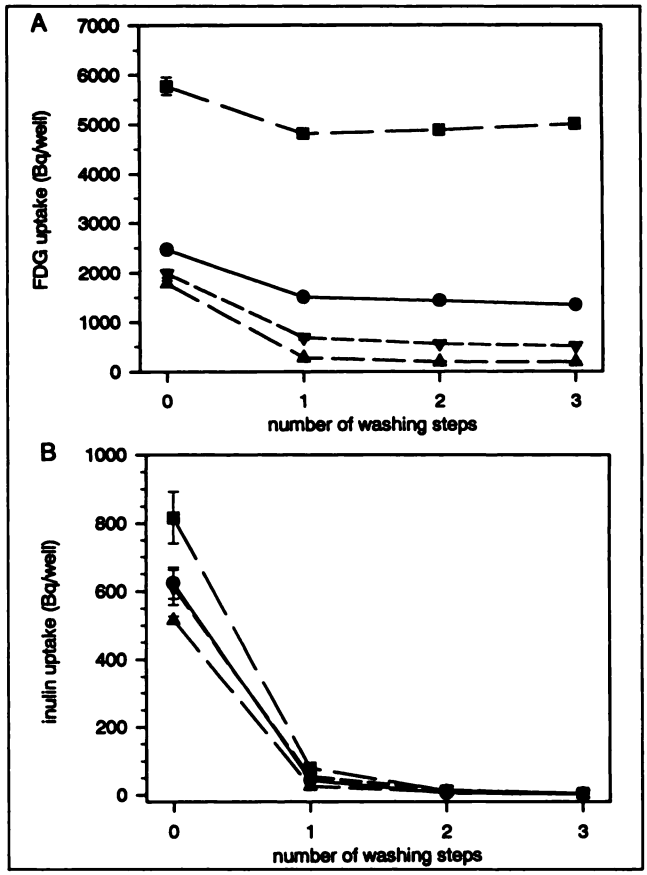

FIGURE 5. Effects of washing on the radioactivity in the cell lysates. The uptake of FDG (A) and inulin (B) was measured in glucose-free medium (squares = $1.58 \cdot 10^6$ cells; circles = $5.54 \cdot 10^5$ cells) or in 0.8-g/liter medium (inverted triangles = $1.58 \cdot 10^6$ cells; triangles = $5.54 \cdot 10^5$ cells). Mean values and s.d. (n = 3).

and 90% ± 1.6% (n = 6) was phosphorylated in cells treated with 100 n*M* dFdC.

DISCUSSION

In vitro assays of FDG uptake give additional and clarifying data to clinical trials. The classic biochemical assay of hexose uptake is performed under glucose-free conditions with either a mixture of radioactive and cold tracer (deoxyglucose or 3-O-methylglucose) with an end concentration of 0.1 or 0.2 m*M* or by use of the radiotracer without any addition of cold material (*7,8,11,19–22*). This procedure may be questioned because of its unphysiologic nature. There is no in vivo situation with a glucose-free environment of tumor cells. The alternative is incubation in a medium with a glucose concentration in the in vivo range of human serum. However, there may be quarrels about the stability of the glucose concentration during the uptake experiment. Furthermore, the difference of a monolayer culture in glucose-containing medium and a monolayer culture in glucose-free medium is not the difference between physiologic and nonphysiologic, i.e., in the artificial context of a cell culture experiment, a procedure is not more

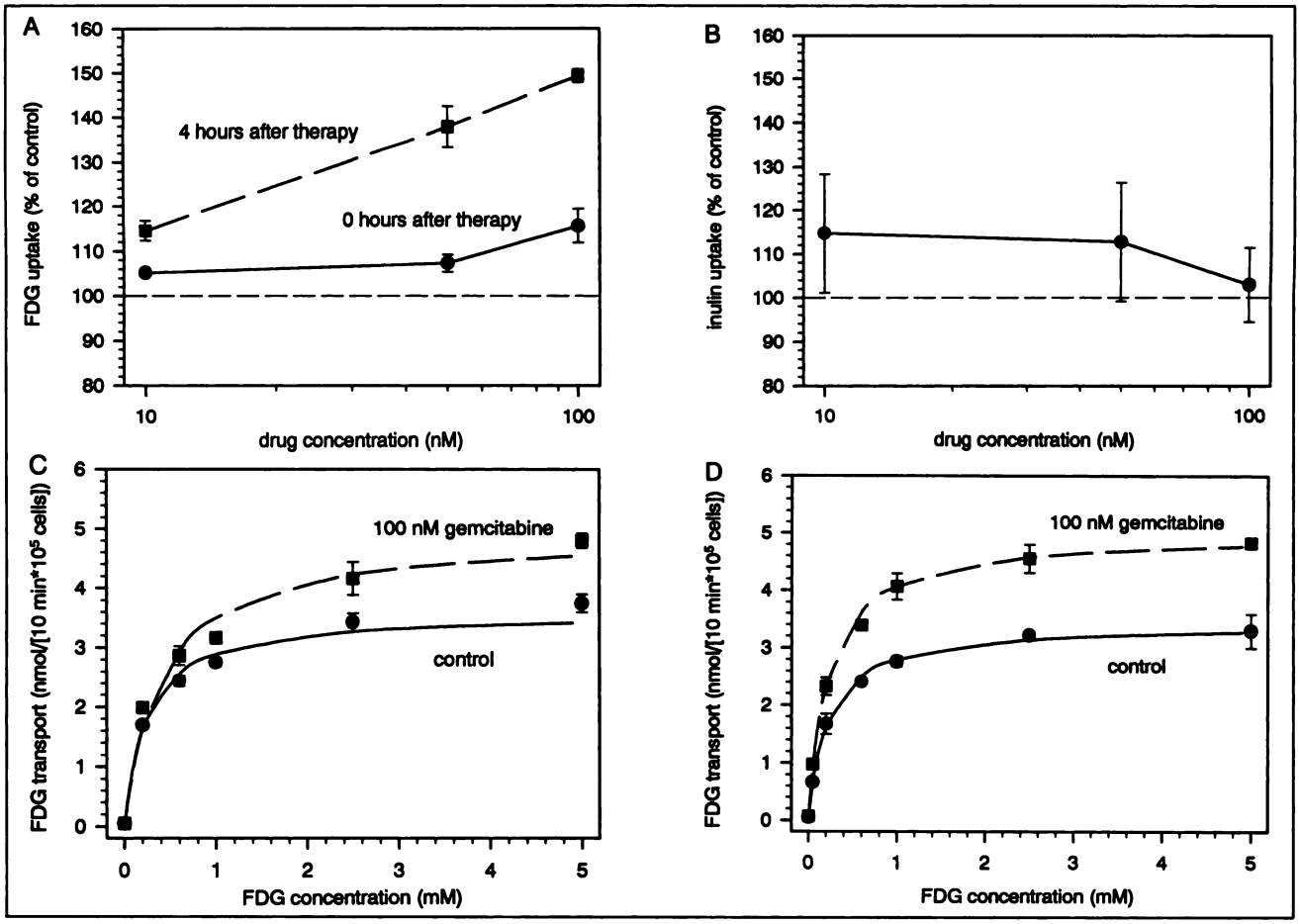

FIGURE 6. FDG uptake after treatment with dFdC. (A) The FDG uptake was measured immediately and 4 hr after therapy with the indicated doses of dFdC. The cell numbers were 2.79 (controls), $2.77 \cdot 10^5$ (10 n*M*), $2.7 \cdot 10^5$ (50 n*M*) and $2.71 \cdot 10^5$ (100 n*M*) immediately after treatment and $3.63 \cdot 10^5$, $3.46 \cdot 10^5$, $3.23 \cdot 10^5$ and $3.18 \cdot 10^5$ cells 4 hr after treatment. (B) No difference in inulin uptake was found between controls and treated cells. Furthermore, V_{max} and K_m for FDG transport (Table 1) were determined in control cells and cells treated with 100 n*M* dFdC immediately ($3.5 \cdot 10^5$ and $3.2 \cdot 10^5$ cells) and 4 hr ($4 \cdot 10^5$ and $3.2 \cdot 10^5$ cells) after treatment (C and D). Mean values and s.d. (n = 3).

"physiologic" as a result only of the presence of glucose in the medium.

A constant decrease in FDG uptake was found with an increasing cell number for the glucose-free (Fig. 2A) and the 0.8-g/liter and the 2-g/liter glucose medium after an incubation period of 60 min (Fig. 2B). This may be due to significant changes in the radiotracer concentration during the incubation time, when the tracer is used at very low-end concentrations (Table 2). The shorter incubation time of 10 min yielded more stable uptake values (Figs. 2C and D).

TABLE 1
Maximum Velocity and Affinity of Fluorodeoxyglucose Transport in Controls and in Cells Treated with 100 nM Gemcitabine Immediately and Four Hours After Treatment (Fit Parameters and Root Mean Square Errors of Estimate)

Condition	V_{max} (nmole/10 min/10^5 cells)	K_m (mM)
Control at 0 hr	3.58 ± 0.14	0.238 ± 0.015
Gemcitabine	4.10 ± 0.24	0.259 ± 0.044
Control at 4 hr	3.42 ± 0.07	0.222 ± 0.015
Gemcitabine	4.99 ± 0.09	0.228 ± 0.015

V_{max} = maximum velocity; K_m = affinity.

The FDG uptake may be influenced by the concentration of D-glucose at the transport level (*19,20,23–25*) and at the phosphorylation level (*19,20,25–28*) because both substances compete for the same transport and kinase system. Because glucose and glucose analogs, such as deoxyglucose and FDG, show different V_{max}s and K_ms for both hexose transport and phosphorylation, with the V_{max} of deoxyglucose being threefold to eightfold lower than the V_{max} of glucose (*23,24*), the glucose content in the medium will decrease faster than the FDG content. Therefore, the glucose-to-FDG ratio shifts to an unpredictable value, which influences the uptake of the radioactive tracer in a way that allows no reliable measurements. From these data, which show a linear decrease in the glucose content in the medium with increasing cell number and incubation

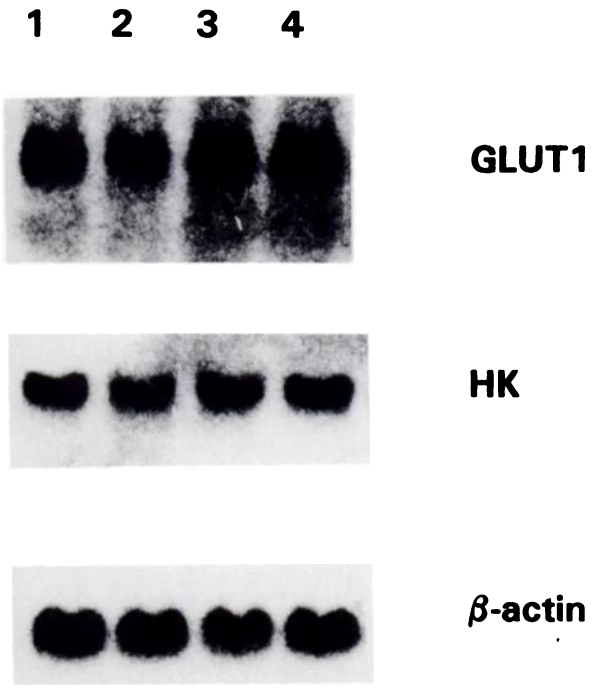

FIGURE 7. Expression of GLUT1, HK and beta-actin in controls (lane 1) and in cells 4 hr after 10 n*M* (lane 2), 50 n*M* (lane 3) and 100 n*M* (lane 4) dFdC. No significant differences in the expression of the GLUT1 and HK were found compared with that in the controls.

time (Fig. 4), it was concluded that, in glucose-containing media, the glucose-to-FDG ratio must be kept at a constant level. This may be done by continuous perfusion of the cells with fresh medium. However, this procedure seems to be technically complicated, and a simpler method may be required. Small cell numbers in large medium volumes may not lead to dramatic alterations in glucose content and may also be an option. However, the use of a procedure that is independent of the medium's glucose content avoids any ambiguities. Stable results and small standard deviations were found in the assay that used an end concentration of 0.1 m*M* FDG and an incubation time of 10 min. Also, no dependence of the FDG transport constants V_{max} and K_m on the cell number was observed in the zero-trans assay (Fig. 3). Therefore, the 0.1-m*M* assay is suggested as a screening test to assess changes in metabolism, e.g., after chemotherapy, and the zero-trans assay as a more sophisticated procedure to obtain data about V_{max} and K_m of the hexose transport system. For both procedures, two washing steps prior to cell lysis are sufficient to eliminate contaminating radioactivity (Fig. 5).

These two optimized procedures were applied to measure the effects of a chemotherapeutic agent. The FDG uptake in treated cells increased, which was stronger at 4 hr after dFdC (Fig. 6A). From the inulin data of this study and from the fact that no significant change in cell volume occurred, unspecific membrane damage was excluded as a possible cause. Similar findings were obtained in studies with doxorubicin (*3*), cisplatin (*29*), bleomycin (*30*) and an ether lipid (*4*) in different cell lines and in mice with Lewis lung carcinomas (*31*). A clinical study revealed an increase in the regional cerebral metabolic rate in brain tumors after chemotherapy, which decreased to the baseline value during the next 4 days (*32*). Furthermore, an increase in glucose transport has been observed as a reaction to cellular stress (*7,8,33–37*).

Glucose transport may be altered by phosphorylation of the transport protein (*7*), decreased degradation (*38*), translocation from intracellular pools to the plasma membrane (*34–36*) or increased expression of the gene (*39*). No difference was found in the mRNA content of GLUT1 or HK (Fig. 7). This indicated that the enhanced FDG uptake

TABLE 2
Radioactivity in the Medium After Incubation

Incubation time	Radioactivity (Bq · 10^4)			
	Cell number	Glucose free	0.8-g Glucose	2-g Glucose
60 min	$1.05 \cdot 10^5$	3.5 ± 0.02	3.67 ± 0.001	3.68 ± 0.001
	$3.67 \cdot 10^5$	2.92 ± 0.013	3.59 ± 0.01	3.65 ± 0.001
	$8.04 \cdot 10^5$	2.31 ± 0.055	3.51 ± 0.003	3.62 ± 0.001
	$11.4 \cdot 10^5$	1.9 ± 0.03	3.4 ± 0.011	3.59 ± 0.002
	$15.1 \cdot 10^5$	1.6 ± 0.06	3.34 ± 0.024	3.56 ± 0.003

Incubation time	Radioactivity (Bq · 10^4)				
	Cell number	Glucose free	0.1 m*M* FDG	0.8-g Glucose	2-g Glucose
10 min	$1.05 \cdot 10^5$	3.6 ± 0.002	3.67 ± 0.004	3.69 ± 0.001	3.69 ± 0.001
	$3.67 \cdot 10^5$	3.48 ± 0.008	3.54 ± 0.004	3.67 ± 0.002	3.68 ± 0.001
	$8.04 \cdot 10^5$	3.16 ± 0.009	3.3 ± 0.003	3.66 ± 0.001	3.67 ± 0.001
	$11.4 \cdot 10^5$	2.92 ± 0.007	3.13 ± 0.023	3.61 ± 0.008	3.66 ± 0.002
	$15.1 \cdot 10^5$	2.74 ± 0.029	3.0 ± 0.008	3.58 ± 0.008	3.65 ± 0.001

after therapy with dFdC is not caused by an increase in the transcription of glycolysis-associated genes. The mechanisms at the post-transcriptional level are phosphorylation, which causes a change in the affinity of the transport protein, decreased degradation or translocation of the protein. Because K_m was not different in controls and treated cells, phosphorylation can be ruled out (Figs. 6C and D and Table 1). The difference in V_{max}, however, suggests that the number of transport proteins is increased. Because a decreased degradation of the glucose carrier has been observed only after transfection of fibroblasts with the oncogene *src* and not as a part of reactions to cellular stress, a redistribution of the protein from intracellular pools to the plasma membrane seems to be more likely. Such reactions have been found in cells exposed to arsenite, calcium ionophore A23187 or 2-mercaptoethanol (*8,33–37*). It was, therefore, suggested that the glucose carrier belongs to the family of stress proteins (*33*) and that a glucose-regulated state may induce resistance to some drugs, such as doxorubicin and etoposide (*37*). Because hypoxia and glucose starvation are two conditions that occur in vivo in tumors, this specific cellular stress response may be observed in a tumor environment.

The phenomenon of increased hexose uptake during or shortly after chemotherapy may be used to increase the sensitivity of tumor cells to chemotherapeutic drugs by disturbance of possible repair mechanisms. Combinations of a chemotherapeutic agent and low doses of a toxic glucose analog, such as deoxyglucose, may help to increase the outcome of therapy while decreasing the side effects. Such combinations have been applied with doxorubicin, etoposide (*37*) and hexadecylphosphocholine (*4*). In the current study, the enhanced uptake of FDG immediately and 4 hr after therapy with dFdC indicated that a combination of dFdC with deoxyglucose leads to increased efficacy. This strategy may also be practicable in vivo. Because PET with FDG has been shown to be valuable for therapeutic monitoring (*31,32,40,41*), it may be applied to measure a therapy-induced increase of FDG uptake shortly after treatment. Thereafter, the PET data may be used for the design of an optimized combination treatment. However, the contribution of nontumor fractions, such as fibroblasts, lymphocytes and macrophages, has to be considered as a possible burden for the exact quantification and interpretation of FDG uptake in tumor tissues.

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