
Early Response of σ -Receptor Ligands and Metabolic PET Tracers to 3 Forms of Chemotherapy: An In Vitro Study in Glioma Cells

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The significant presence of nontumor cell populations within tumors can complicate the assessment of in vivo tumor metabolism during therapy. To more clearly define the impact of cytotoxic agents, we compared early changes in the uptake of 6 PET tracers in cultured glioma cells. Doxorubicin (1 μ mol/L), cisplatin (10 μ mol/L), and 5-fluorouracil (10 mmol/L) were selected to target different aspects of cellular metabolism.

Methods: The tracers were 2 extracellular σ -receptor ligands, ¹⁸F-FE-SA5845 (nonsubtype selective) and ¹¹C-SA4503 (σ -1), the nucleoside 3'-deoxy-3'-¹⁸F-fluorothymidine (¹⁸F-FLT), ¹¹C-choline, ¹¹C-methionine, and ¹⁸F-FDG. C6 glioma cells were grown as monolayers and exposed to cytotoxic agents at concentrations at least 1 order of magnitude higher than the concentration for 50% growth inhibition of this cell line. Effects on cellular parameters were measured after 0, 1, 2, 3, 4, and 24 h.

Results: All treatments resulted in a decline in cell numbers within 24 h. The binding of the σ -ligands ¹¹C-SA4503 and ¹⁸F-FE-SA5845 and the uptake of ¹¹C-choline (normalized for the number of viable cells) were strongly increased. The uptake of ¹⁸F-FDG showed little change, and cellular accumulation of ¹⁸F-FLT and ¹¹C-methionine was decreased. Uptake of ¹⁸F-FLT and ¹¹C-methionine was related to the fraction of cells in S-phase, but not under all conditions: (a) doxorubicin caused a more rapid decline in ¹⁸F-FLT uptake than in the S-phase fraction because of depletion of cellular adenosine triphosphate, and (b) cisplatin inhibited the transport of ¹¹C-methionine across the tumor cell membrane. **Conclusion:** Increased binding of σ -ligands and an increased uptake of ¹¹C-choline after chemotherapy may reflect active membrane repair in damaged cells. ¹⁸F-FLT and ¹¹C-methionine behaved as proliferation markers. However, the accumulation of ¹⁸F-FDG reflected not the proliferation rate but, rather, the number of viable cells per well.

Key Words: σ -receptor ligands; 3'-deoxy-3'-fluorothymidine; doxorubicin; cisplatin; 5-fluorouracil

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Although the most widely used radiopharmaceutical in clinical oncology is ¹⁸F-FDG, this glucose analog is not useful under all circumstances. High physiologic uptake of glucose in the healthy brain and strong accumulation of radioactivity in the urinary bladder limit the use of ¹⁸F-FDG for tumor detection in the central nervous system (1) and in the lower part of the abdomen (2). A high rate of false-positive lesions further restricts the use of ¹⁸F-FDG for certain applications (3,4). ¹⁸F-FDG is not tumor cell specific and it can also accumulate in other cells—for example, active macrophages (4). This lack of specificity is a pitfall in the monitoring of therapy control with ¹⁸F-FDG PET after chemotherapy (5). Thus, there is opportunity for the development of alternative imaging agents that lack some of the disadvantages of ¹⁸F-FDG.

¹¹C- and ¹⁸F-labeled amino acids, nucleosides, and choline have been developed as radiopharmaceuticals to target elevated synthesis of proteins, DNA, and biomembranes in malignant tissue (6,4,7–9). σ -Receptor ligands, which have been proposed as novel radiopharmaceuticals for tumor imaging, are also proliferation markers (10). These radio-tracers track the synthesis of cell membrane lipids (11).

Oncologic tracers can be used not only for tumor detection but also for evaluation of the tumor response to treatment. Metabolic changes induced by chemotherapy precede the morphologic changes. Radiolabeled compounds that track different aspects of cell metabolism may show different uptake kinetics after therapy. The uptake of an ideal tracer would show a rapid change in responding tumors and the magnitude of this change would correspond to the final therapy outcome.

Various radiopharmaceuticals have been examined, comparatively, as early response indicators of chemotherapy. Sato et al. (12) measured uptake changes of 2 nucleosides (¹⁸F-5-fluoro-2'-deoxyuridine, ¹⁴C-thymidine), 1 amino acid (¹⁴C-methionine), and a glucose analog (³H-deoxyglucose) in rat glioma after in vivo chemotherapy with 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea (ACNU). Schaidt et al. (13) monitored the uptake of 2

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amino acids (^3H - α -aminoisobutyric acid and L-[^{14}C -methyl]methionine), a nucleoside (^3H -thymidine), and a glucose analog (^{18}F -FDG) in a human colon carcinoma cell line during in vitro chemotherapy with dinaline. Takeda et al (14) measured sequential changes in DNA synthesis, glucose use, protein synthesis, and peripheral benzodiazepine receptor density in C6 tumors within rat brain in vivo during chemotherapy with nitrosoureas. To the best of our knowledge, treatment responses of σ -receptor ligands have never been compared with those of established PET tracers (^{18}F -FDG, 3'-deoxy-3'-fluorothymidine [^{18}F -FLT], ^{11}C -methionine) and of ^{11}C -choline. Therefore, we decided to measure changes in the uptake of the σ -1 ligand ^{11}C -SA4503 and the nonsubtype-selective σ -ligand ^{18}F -FE-SA5845, ^{18}F -FLT, ^{11}C -choline, ^{11}C -methionine, and ^{18}F -FDG during 3 forms of chemotherapy in vitro.

A considerable (and unknown) fraction of the total mass of in vivo tumors consists of normal cells. Human breast cancers have been reported to contain 19%–64% of macrophages (15) and up to 10% of lymphocytes (16). Thus, nontumor cells may contribute significantly to the radioactive signal being measured and their presence complicates the assessment of changes in tumor metabolism during therapy. In vitro models contain only tumor cells. Changes in tracer uptake in these models are a more direct reflection of metabolic impact within tumor cells. Thus, in vitro data can be more easily interpreted than results from in vivo studies.

For our own in vitro chemotherapy model we used cisplatin, doxorubicin, and 5-fluorouracil, compounds that interact with DNA (17), topoisomerase II (18), and thymidylate synthetase (19), respectively. The agents were administered to C6 glioma cells in concentrations that were >1 order of magnitude greater than the concentration for 50% growth inhibition (GIC_{50}) (20). The C6 line was chosen because it had also been used in our previous work on σ -ligands (21,22).

MATERIALS AND METHODS

Culture Media and Drugs

Cisplatin (cis-diammineplatinum(II) dichloride) was obtained from Sigma. Fluracedyl (5-fluorouracil solution, 50 mg/mL) and doxorubicin were purchased from Pharmachemie. Dulbecco's minimum essential medium (DMEM), fetal calf serum (FCS), and trypsin were from Invitrogen.

Radiopharmaceuticals

^{11}C -Methionine, ^{11}C -choline, ^{18}F -FLT, and ^{18}F -FDG were prepared by standard procedures reported in the literature (22). ^{18}F -FE-SA5845 and ^{11}C -SA4503 were prepared by reaction of ^{18}F -fluoroethyl tosylate and ^{11}C -methyl iodide, respectively, with the appropriate 4-*O*-methyl compound (23). All radiochemical purities were >95%.

Cell Culture and Treatment with Cytostatic Agents

C6 rat glioma cells obtained from the American Type Culture Collection were cultured in monolayers in DMEM supplemented with 5% FCS in a humidified atmosphere of 5% CO_2 /95% air at

37°C. Before each experiment, the cells were seeded in 12-well plates (Costar) with 1.3 mL of DMEM supplemented with 5% FCS per well. After 24 h at 37°C, monolayers were established. Cytotoxic agents (final concentration in culture medium: 1 $\mu\text{mol/L}$ doxorubicin, 10 $\mu\text{mol/L}$ cisplatin, 10 mmol/L 5-fluorouracil) were then added to the wells 24, 4, 3, 2, and 1 h before addition of radiotracer.

Incubation with Radiotracer

Experiments were performed in quadruplicate. Studies of radiotracer uptake were performed in monolayers grown at the bottom of 12-well plates. At time zero, 2 MBq of a radiotracer (^{11}C -methionine, ^{11}C -choline, ^{11}C -SA4503, ^{18}F -FDG, ^{18}F -FLT, or ^{18}F -FE-SA5485) in 50 μL of saline were added to each well. At the end of incubation (60 min), the medium was quickly removed and the monolayer was washed 3 times with chilled phosphate-buffered saline (PBS). Cells were then treated with 0.2 mL of trypsin. When the cells had detached from the bottom of the well (within 5 min), 1 mL of DMEM was added to stop the proteolytic action. Cell clumps were removed by repeated (at least 10-fold) pipetting of the trypsin/DMEM mixture. Radioactivity in the cell suspension (1.2 mL) was assessed using a γ -counter (Compu-gamma 1282 CS; LKB-Wallac). A 50- μL sample of the suspension was mixed with 50 μL of trypan blue (0.4% solution in PBS) and used for cell counting. Cell numbers were manually determined, using a phase-contrast microscope (Olympus), a Bürker bright-line chamber (depth, 0.1 mm; 0.0025-mm² squares), and a hand-tally counter.

Propidium Iodide (PI) Staining and Flow Cytometric Analysis

C6 cells were grown and harvested as described; 2 wells from a 12-well plate were pooled. The cell samples were washed twice with PBS containing 5 mmol/L MgCl_2 . After each washing step, a cell pellet was collected by short centrifugation (5 min, 2,000 rpm; Hettich tabletop centrifuge). The washed cells were fixed in 2 mL of 80% ethanol/acetone (1:1, v/v) at 4°C for at least 18 h. Fixed cells were washed once with 5 mL PBS containing 5 mmol/L MgCl_2 and the pellet was resuspended in 20 μL of DNase-free RNase A (Sigma) and incubated for 30 min at 37°C. At the end of the incubation, 400 μL PI (Sigma) was added (a solution of 10 $\mu\text{g/mL}$ in PBS with 5 mmol/L MgCl_2) and the mixture was stored in the refrigerator (4°C) for at least 18 h. Cellular DNA content was determined with a FACSCalibur (Becton Dickinson). Data were plotted using CellQuest software (Becton Dickinson); at least 5,000 events were analyzed for each sample.

Thymidine Kinase Assay

C6 cells were grown and harvested as described; 4 wells from a 12-well plate were pooled. A cell pellet was collected by a short centrifugation (as above). The pellet was resuspended in 0.5 mL of ice-cold lysis buffer (20 mmol/L Tris-HCl, pH 8.1, 40 mmol/L KCl, 1 mmol/L ethylenediaminetetraacetic acid, 10% glycerol, 1 mmol/L dithiothreitol (DTT), 1 mmol/L phenylmethylsulfonyl-fluoride, 1 $\mu\text{g/mL}$ aprotinin). The cells were lysed by repeated sonication at 0°C. Cell and membrane fragments were removed from the lysate by centrifugation (Hettich Mikrofuge, 5 min, 12,000 $\times g$).

The enzyme reaction mixture (ERM) contained 80 mmol/L potassium phosphate buffer, pH 7.6, 160 mmol/L KCl, 100 mmol/L NaF, 4 mg/mL bovine serum albumin, 20 mmol/L adenosine triphosphate (ATP)- MgCl_2 , and 4 mmol/L DTT. Equal volumes of

cell lysate (0.5 mL) and ERM (0.5 mL) were mixed and incubated with 2 MBq ^{18}F -FLT in a closed glass vial for 60 min at 37°C. Two samples (50 μL) were taken from each vial and pipetted onto Whatman DE-81 filters. One filter remained untreated; the other filter was washed 3 times with a 4 mmol/L ammonium formate solution and 3 times with ethanol. Radioactivity on the filters was determined with a γ -counter (as above). Activity on the washed filter represented ^{18}F -FLT phosphates, whereas the activity on the untreated filter represented ^{18}F -FLT phosphates plus unreacted ^{18}F -FLT. A blank incubation (0.5 mL lysis buffer without C6 cells) was run in parallel during each experiment.

Preliminary experiments indicated that the thymidine kinase assay was linear for incubation times up to 90 min. Therefore, an incubation time of 60 min was chosen in subsequent experiments. Radioactivity in blank incubations was <3% of that in cell lysates after 60 min.

ATP Assay

Cellular ATP was measured with a kit based on firefly (*Photinus pyralis*) luciferase (ATPlite Luminescence ATP Detection Assay System; Perkin-Elmer). The bioluminescence was quantified using a Lumicount microplate luminometer (Packard Instruments Co.). A reaction blank and a standard curve with 7 concentrations of ATP were run in parallel. The assay was linear over a large range of ATP concentrations (5×10^{-9} to 10^{-6} mol/L) and of cell numbers in the assay. Samples were diluted to ensure that the concentration of ATP was within the range of the calibration curve.

Statistics

Differences between groups were examined using 1-way ANOVA. A P value < 0.05 was considered statistically significant.

RESULTS

Cell Number

The number of viable cells per well decreased as a result of chemotherapy. The 3 cytostatic agents inhibited cellular proliferation with different time courses (Fig. 1). The effect of doxorubicin (1 $\mu\text{mol/L}$) was relatively rapid. A statistically significant cell loss was already observed after 3 h of incubation, and a progressive decline (to $64\% \pm 2\%$ of control; $P < 0.0001$) occurred between 3 and 24 h. Cisplatin (10 $\mu\text{mol/L}$) caused no significant decrease in cell numbers during the initial 4 h, but a highly significant cell loss (to $63\% \pm 3\%$ of control; $P < 0.0001$) occurred between 4 and 24 h. Incubation of cells with 5-fluorouracil (10 mmol/L) caused a very rapid cell loss. Cell numbers decreased already within 1 h, and a very strong decrease (to $15\% \pm 2\%$ of control; $P < 0.0001$) was observed after 24 h.

Fraction of Cells in S-phase

The 3 cytostatic agents also had different effects on the fraction of cells in S-phase (Fig. 1). During the first 4 h of treatment, all compounds caused a slight increase in this parameter. However, whereas doxorubicin and 5-fluorouracil caused a significant decrease in the relative numbers of cells in S-phase after 24 h, cisplatin had the opposite effect (Fig. 1). The S-phase fraction was changed from $43\% \pm 1\%$ at time zero to $14\% \pm 3\%$ (doxorubicin; $P < 0.0001$),

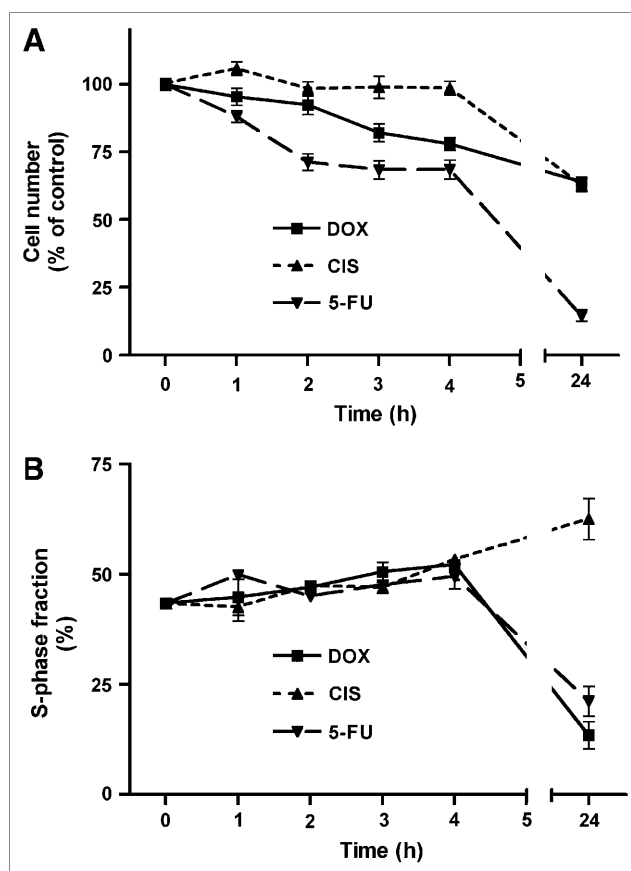


FIGURE 1. Decreases in cell number (A) and changes in S-phase fraction (B) after onset of chemotherapy. Data are plotted as mean \pm SEM. DOX = doxorubicin; CIS = cisplatin; 5-FU = 5-fluorouracil.

$63\% \pm 5\%$ (cisplatin; $P < 0.0005$), and $21\% \pm 3\%$ (5-fluorouracil; $P = 0.0001$) after 24 h of treatment.

^{11}C -SA4503

Cytostatic agents caused an increase in the binding of the σ -1 ligand ^{11}C -SA4503 to C6 cells (Fig. 2). A statistically significant increase of tracer binding was already noted within 1 h of treatment with doxorubicin or 5-fluorouracil and within 2 h of incubation with cisplatin. After 24 h of treatment, SA4503 uptake per cell was increased to $168\% \pm 9\%$ (doxorubicin; $P = 0.0005$), $206\% \pm 20\%$ (cisplatin; $P = 0.002$), and $272\% \pm 7\%$ of control (5-fluorouracil; $P < 0.0001$).

^{18}F -FE-SA5845

Cellular binding of the nonsubtype selective σ -ligand ^{18}F -FE-SA5845 responded less strongly than that of the σ -1 agonist ^{11}C -SA4503 to chemotherapy. During 24 h of treatment of cells with doxorubicin, the binding of ^{18}F -FE-SA5845 per cell did not show any change (Fig. 2). However, a significant increase of ^{18}F -FE-SA5845 binding was observed after 3, 4, and 24 h of treatment with cisplatin and after 24 h of incubation with 5-fluorouracil. ^{18}F -FE-SA5845 binding was increased to $157\% \pm 5\%$ (cisplatin;

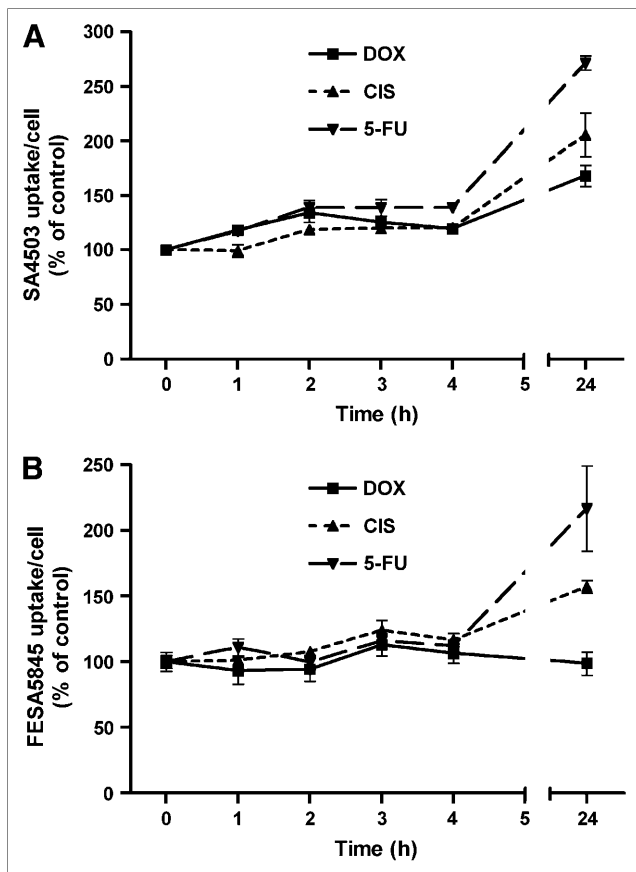


FIGURE 2. Cellular binding of σ -ligands ^{11}C -SA4503 (A) and ^{18}F -FE-SA5845 (B) during 24 h of chemotherapy. Data are plotted as mean \pm SEM. DOX = doxorubicin; CIS = cisplatin; 5-FU = 5-fluorouracil.

$P = 0.0001$) and $217\% \pm 32\%$ of control (5-fluorouracil; $P = 0.01$) after 24 h of treatment.

^{11}C -Choline

During the initial 4 h of treatment with doxorubicin and cisplatin, uptake of ^{11}C -choline per viable cell remained constant, but a rapid increase (within 1 h) was induced by 5-fluorouracil (Fig. 3). ^{11}C -Choline uptake was significantly increased by all treatments after 24 h, to $132\% \pm 7\%$ (doxorubicin; $P < 0.01$), $164\% \pm 10\%$ (cisplatin; $P < 0.001$), and $203\% \pm 16\%$ of control (5-fluorouracil; $P < 0.001$).

^{18}F -FDG

During treatment of C6 cells with doxorubicin and cisplatin, uptake of ^{18}F -FDG per cell remained constant throughout the entire study period (Fig. 3). After treatment with 5-fluorouracil, ^{18}F -FDG uptake remained constant during the initial 4 h. However, this cytostatic agent induced a small decrease in ^{18}F -FDG uptake after 24 h (to $79\% \pm 5\%$ of control, $P < 0.005$; Fig. 3).

^{11}C -Methionine

The uptake of ^{11}C -methionine per cell was depressed by chemotherapy, although this was a relatively late effect (Fig. 4). During the initial 4 h of treatment with all cyto-

static agents, cellular uptake of the amino acid remained constant. A statistically significant decline occurred after 24 h, to $62\% \pm 4\%$ (doxorubicin; $P < 0.002$), $91\% \pm 3\%$ (cisplatin; $P = 0.05$), and $71\% \pm 3\%$ of control (5-fluorouracil, $P < 0.005$).

^{18}F -FLT

Uptake of the nucleoside ^{18}F -FLT showed the strongest response to chemotherapy of all studied PET tracers (Fig. 4). A rapid and significant decrease (within 1 h) was observed after treatment of cells with doxorubicin; after 24 h of treatment ^{18}F -FLT uptake was almost completely suppressed ($1.3\% \pm 0.2\%$ of control; $P < 0.0001$). A less rapid decrease occurred during chemotherapy with cisplatin. ^{18}F -FLT uptake was not significantly altered during the initial 4 h of cisplatin treatment, but a strong decrease (to $49\% \pm 4\%$ of control; $P < 0.0001$) occurred after 24 h. Incubation of cells with 5-fluorouracil had a biphasic effect on cellular ^{18}F -FLT uptake. During the initial 4 h of treatment, uptake of the nucleoside was significantly increased (from $100\% \pm 6\%$ to $143\% \pm 10\%$; $P = 0.01$), but it was strongly depressed after 24 h (to $32\% \pm 7\%$ of control; $P = 0.0001$).

Activity of Cellular Thymidine Kinase

The activity of cellular thymidine kinase (normalized for the number of viable cells) showed a similar pattern of

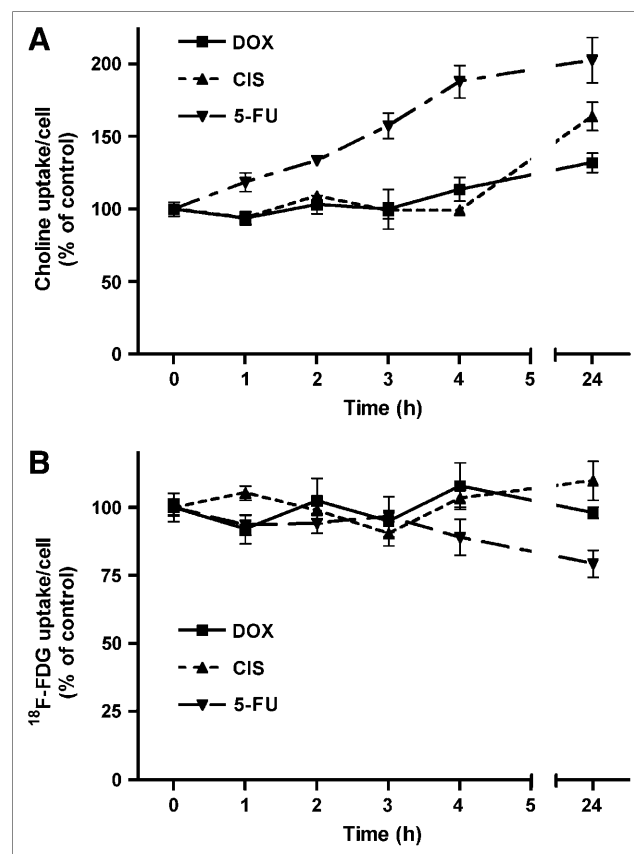


FIGURE 3. Uptake of ^{11}C -choline (A) and of ^{18}F -FDG per cell (B) during 24 h of chemotherapy. Data are plotted as mean \pm SEM. DOX = doxorubicin; CIS = cisplatin; 5-FU = 5-fluorouracil.

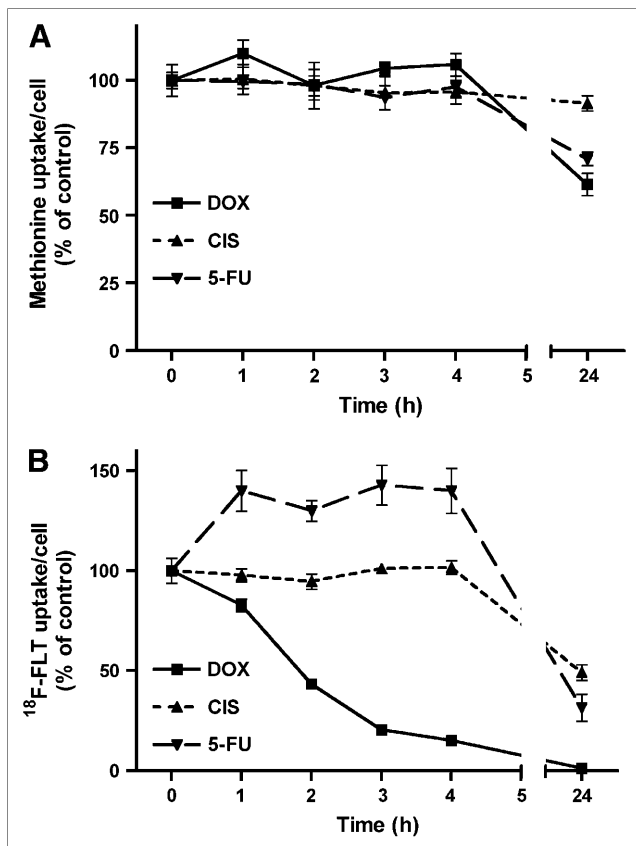


FIGURE 4. Uptake of ^{11}C -methionine (A) and of ^{18}F -FLT per cell (B) during 24 h of chemotherapy. Data are plotted as mean \pm SEM. On some curves, error bars are within size of symbols and, therefore, are not visible. DOX = doxorubicin; CIS = cisplatin; 5-FU = 5-fluorouracil.

changes after 24 h of treatment with cytostatic agents as the fraction of cells in S-phase (Fig. 5). Doxorubicin and 5-fluorouracil caused a significant decrease in cellular thymidine kinase activity after 24 h ($P < 0.0002$ in both cases), whereas cisplatin caused an increase ($P < 0.005$; Fig. 5). There was much variability in the enzyme data after 2–4 h of treatment. 5-Fluorouracil induced a transient increase in enzyme activity during this period ($P < 0.0001$), followed by a subsequent decline ($P < 0.0002$; Fig. 5).

Cellular ATP Content

The amount of ATP per cell was decreased during 24 h of treatment with doxorubicin (to $60\% \pm 2\%$ of control; $P < 0.01$) but was virtually unchanged after treatment with cisplatin and 5-fluorouracil (Fig. 5).

DISCUSSION

Cell Number

On the basis of the doses used, we expected a reduction in cell numbers after treatment of C6 cells with cytostatic agents. The dose of doxorubicin that we administered was >7 times higher than the GIC_{50} for this cell line (24). Doses of cisplatin and 5-fluorouracil were also an order of

magnitude higher than those required for 50% growth inhibition (20,25). The reduction in the number of viable C6 cells that we observed after 24 h of treatment with cytostatic agents (Fig. 1) is very similar to values reported in the literature (20,26).

S-Phase Fraction

In our in vitro chemotherapy model, cytostatic agents had different effects on the fraction of cells in S-phase after 24 h. Doxorubicin and 5-fluorouracil decreased the S-phase fraction, in contrast to cisplatin (Fig. 1). Reports in the literature indicate that doxorubicin and 5-fluorouracil induce $\text{G}_2\text{-M}$ arrest (27) and combined G_1/G_2 arrest (28), respectively, with corresponding decreases in the fraction of cells in S-phase. In contrast, cisplatin initially blocks C6 cells in the S-phase, resulting in a transient increase in the S-phase fraction. Only after longer periods of treatment with cisplatin (≥ 3 d), a fair number of cells are blocked at the premitotic G_2 -phase of the cell cycle (26).

Tracer Response to Treatment

Three responses of tracer uptake to chemotherapy were observed in the current study: (a) increased uptake (σ -ligands ^{11}C -SA4503 and ^{18}F -FE-SA5845, ^{11}C -choline;

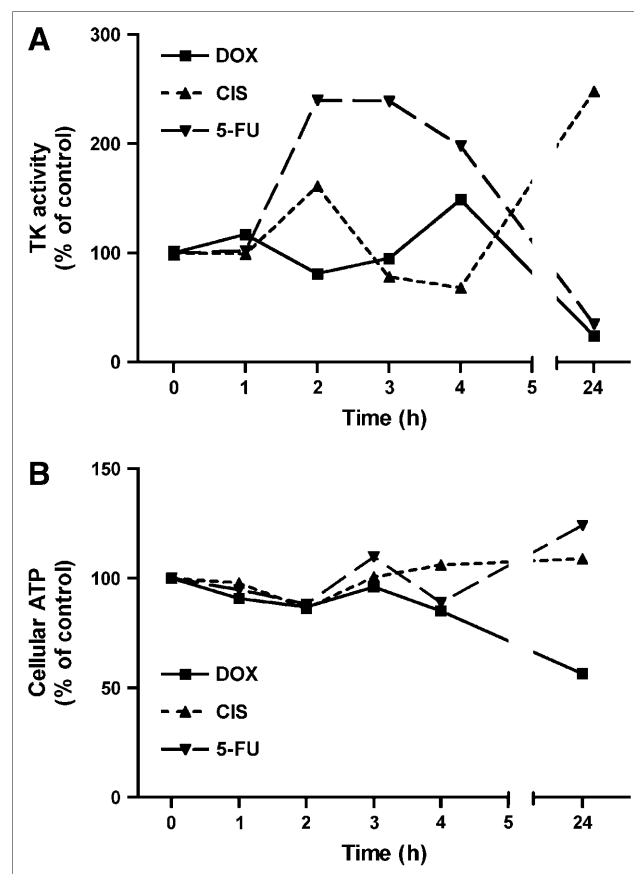


FIGURE 5. Effect of chemotherapy on activity of cellular thymidine kinase (TK) (A) and on cellular ATP content (B). Data are plotted as mean \pm SEM. Error bars are within size of symbols. DOX = doxorubicin; CIS = cisplatin; 5-FU = 5-fluorouracil.

Figs. 2 and 3), (b) relatively little change in cell-specific uptake (^{18}F -FDG; Fig. 3), and (c) decreased uptake (^{11}C -methionine, ^{18}F -FLT; Fig. 4).

To our knowledge, there are no published reports about changes in uptake of σ -ligands in tumors or tumor cells after chemotherapy. Our data indicate that the cellular uptake of these tracers is initially increased (Figs. 2 and 3). The increased uptake of σ -ligands (and of ^{11}C -choline) may be related to an enhancement of lipid synthesis caused by repair mechanisms (11). ^{11}C -SA4503 and ^{18}F -FE-SA5845 have different subtype selectivities and, therefore, may show different tracer kinetics. ^{11}C -SA4503 binds to the σ -1 subtype only, whereas ^{18}F -FE-SA5845 has considerable affinity to both σ -1 and σ -2 receptors (23). The stronger increase of ^{11}C -SA4503-uptake than of ^{18}F -FE-SA5845-uptake after chemotherapy (Fig. 2) suggests that the σ -1 and σ -2 receptor populations respond differently to treatment with cytostatic agents.

A study in the literature has indicated that radiolabeled choline is not very suitable for evaluation of cytotoxic effects as the uptake of ^3H -choline (and also of ^{18}F -FDG) in prostate tumor cells did not show any decrease after treatment of the cells with 2-methoxyestradiol (29). Our own data indicate that uptake of ^{11}C -choline in C6 cells is increased after 24 h of antitumor therapy in contrast to that of the proliferation marker ^{18}F -FLT (Figs. 3 and 4).

A relatively small response of ^{18}F -FDG uptake to chemotherapy, compared with the response of radiolabeled nucleosides, has often been reported. In murine RIF-1 tumors, the 5-fluorouracil-induced reduction in ^{18}F -FLT uptake was more rapid and significantly more pronounced than that of ^{18}F -FDG (30). Similar observations were made in a hormone-responsive rat tumor model, using radioiodinated 5-iodo-2'-deoxyuridine and ^3H -deoxyglucose (31). In OSC-1 tumor cells grown in vitro, cisplatin induced a significant and dose-dependent reduction in the uptake of ^{18}F -FLT, whereas the uptake of ^{18}F -FDG was unchanged after 24 h (32). Fleischmannova et al. (33) observed a 54% decline in the uptake of ^3H -thymidine in C6 rat glioma cells after 20 h of treatment with 10 μg cisplatin/mL, close to the value that we observed for ^{18}F -FLT (Fig. 4).

Because the uptake of ^{18}F -FDG per cell showed little change during most forms of chemotherapy (Fig. 3), ^{18}F -FDG appeared to trace the number of viable cells per well rather than the proliferative rate of the cells. Higashi et al. (34) reached the same conclusion in a human ovarian adenocarcinoma cell line. ^{18}F -FDG uptake was largely unchanged as carcinoma cells progressed through the growth cycle, but cellular ^3H -thymidine uptake decreased when the cells progressed from the lag phase to the plateau phase. The authors concluded that, in their cell line, ^{18}F -FDG measured a substantially different parameter (viable cell number) than ^3H -thymidine (proliferative rate). Rasey et al. (35) reported a much greater variation in the uptake of ^{18}F -FLT than of ^{14}C -deoxyglucose as a function of the growth rate in human lung carcinoma cells.

Sato et al. (12) studied changes in the uptake of 2 radiolabeled nucleosides, one amino acid and a glucose analog in a rat glioma (KEG-1) after chemotherapy. They observed an immediate, sharp fall in nucleoside uptake (^{18}F -5-fluoro-2'-deoxyuridine and ^{14}C -thymidine), a moderate fall in the uptake of ^{14}C -methionine and, only after a long interval (>1 wk), any decline in the uptake of ^3H -deoxyglucose after treatment of the animals with ACNU. These findings are very similar to our own in vitro observations (Figs. 3 and 4).

S-Phase Fraction and Tracer Uptake

We observed a significant correlation between the uptake of ^{11}C -methionine after treatment of cells with doxorubicin or 5-fluorouracil and the fraction of cells in S-phase ($n = 11$; $r = 0.94$; $P < 0.0001$). However, this correlation did not hold for ^{11}C -methionine uptake after cisplatin treatment. The increase in the S-phase fraction after cisplatin treatment was not accompanied by an increased cellular uptake of ^{11}C -methionine (compare Figs. 1 and 4). The aberrant behavior of ^{11}C -methionine uptake after cisplatin treatment can be attributed to the fact that cisplatin inhibits not only cell division but also the transport of amino acids across the tumor cell membrane. This side effect of cisplatin treatment has been noted previously, using ^{14}C -methionine and L1210 murine leukemia cells (36) or C6 and P388 tumors grown in rats in vivo (37,38).

^{18}F -FLT uptake after 0, 1, 2, 4, and 24 h of treatment of cells with 5-fluorouracil and after 24 h of treatment with doxorubicin also showed a significant relationship with the fraction of cells in S-phase ($n = 7$; $r = 0.99$; $P < 0.0001$). This observation is in accordance with studies in the literature that reported close, or fairly close, correlations between the fraction of tumor cells in S-phase and the cellular uptake of ^{18}F -FLT (35,39). However, we did not observe any correlation between ^{18}F -FLT uptake and the S-phase fraction after treatment of C6 cells with cisplatin or after 1, 2, 3, or 4 h of incubation with doxorubicin. Under such conditions, uptake of the nucleoside was reduced in contrast to the S-phase fraction, which remained high (compare Figs. 1 and 4). Cellular trapping of ^{18}F -FLT is dependent on the activity of thymidine kinase 1 (TK1) (35,40). The activity of this enzyme is determined not only by the fraction of cells in S-phase and the corresponding level of TK1 expression but also by the availability of the cofactor ATP (30). Decreased trapping of ^{18}F -FLT in the presence of a continuing high expression of the TK1 protein may occur in tumor cells when cellular ATP levels fall (30). A bioluminescence assay that we performed indicated that cellular ATP content was decreased after treatment of C6 cells with doxorubicin but not after treatment with cisplatin or 5-fluorouracil (Fig. 5). Thus, the effect of doxorubicin on ^{18}F -FLT uptake may be related to depletion of the cellular ATP pool in the presence of the cytostatic agent, but the mechanism of the cisplatin effect is as yet unknown. Cisplatin may interfere not only with

amino acid transport but also with the transport of nucleosides across the tumor cell membrane (35).

No correlation was observed between the cellular uptake of ^{11}C -choline, ^{11}C -SA4503, ^{18}F -FDG, or ^{18}F -FE-SA5845 and the fraction of cells in S-phase under the conditions of this study.

CONCLUSION

The 6 PET tracers that we studied showed different uptake kinetics after therapy. Increased binding of σ -ligands and an increased uptake of ^{11}C -choline may reflect active membrane repair in damaged cells. Uptake of ^{18}F -FLT and ^{11}C -methionine showed a decline; these tracers acted as proliferation markers. Accumulation of ^{18}F -FDG reflected not proliferation rate, but the number of viable cells per well.

Cellular uptake of the nucleoside ^{18}F -FLT and binding of the σ -1 receptor ligand ^{11}C -SA4503 showed the most rapid changes after the onset of chemotherapy. On the basis of these data from an in vitro model, ^{18}F -FLT appears more suitable as an early predictor of therapeutic response than ^{18}F -FDG. If rapid increases in the binding of ^{11}C -SA4503 are also observed in vivo and are related to the final therapy outcome, ^{11}C -SA4503 may be useful for therapy monitoring and may provide information complementary to ^{18}F -FLT.

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REFERENCES

1. Weber WA, Avril N, Schwaiger M. Relevance of positron emission tomography (PET) in oncology. *Strahlenther Onkol.* 1999;175:356–373.
2. De Jong IJ, Pruim J, Elsinga PH, Vaalburg W, Mensink HJ. Visualization of prostate cancer with ^{11}C -choline positron emission tomography. *Eur Urol.* 2002; 42:18–23.
3. Strauss LG. Fluorine-18 deoxyglucose and false-positive results: a major problem in the diagnostics of oncological patients. *Eur J Nucl Med.* 1996;23: 1409–1415.
4. Kubota K. From tumor biology to clinical PET: a review of positron emission tomography (PET) in oncology. *Ann Nucl Med.* 2001;15:471–486.
5. Lorenzen J, de Wit M, Buchert R, Igel B, Bohuslavizki KH. Granulation tissue: pitfall in therapy control with F-18-FDG PET after chemotherapy. *Nuklearmedizin.* 1999;38:333–336.
6. Kubota K, Yamada K, Fukada H, et al. Tumor detection with carbon-11-labelled amino acids. *Eur J Nucl Med.* 1984;9:136–140.
7. Shields AF, Lim K, Grierson J, Link J, Krohn KA. Utilization of labeled thymidine in DNA synthesis: studies for PET. *J Nucl Med.* 1990;31:337–342.
8. Shields AF, Grierson JR, Dohmen BM, et al. Imaging proliferation in vivo with [F-18]FLT and positron emission tomography. *Nat Med.* 1998;4:1334–1336.
9. Shinoura N, Nishijima M, Hara T, et al. Brain tumors: detection with C-11 choline PET. *Radiology.* 1997;202:497–503.
10. Wheeler KT, Wang LM, Wallen CA, et al. Sigma-2 receptors as a biomarker of proliferation in solid tumours. *Br J Cancer.* 2000;82:1223–1232.
11. Hayashi T, Su TP. The potential role of sigma-1 receptors in lipid transport and lipid raft reconstitution in the brain: implication for drug abuse. *Life Sci.* 2005; 77:1612–1624.
12. Sato K, Kameyama M, Ishiwata K, Katakura R, Yoshimoto T. Metabolic changes of glioma following chemotherapy: an experimental study using four PET tracers. *J Neurooncol.* 1992;14:81–89.
13. Schaidt H, Haberkorn U, Berger MR, Oberdorfer F, Morr I, Van Kaick G. Application of α -aminoisobutyric acid, L-methionine, thymidine and 2-fluoro-2-deoxy-D-glucose to monitor effects of chemotherapy in a human colon carcinoma cell line. *Eur J Nucl Med.* 1996;23:55–60.
14. Takeda N, Diksic M, Yamamoto YL. The sequential changes in DNA synthesis, glucose utilization, protein synthesis, and peripheral benzodiazepine receptor density in C6 brain tumors after chemotherapy to predict the response of tumors to chemotherapy. *Cancer.* 1996;77:1167–1179.
15. Steele RJ, Brown M, Eremin O. Characterisation of macrophages infiltrating human mammary carcinomas. *Br J Cancer.* 1985;51:135–138.
16. Whitford P, Mallon EA, George WD, Campbell AM. Flow cytometric analysis of tumour infiltrating lymphocytes in breast cancer. *Br J Cancer.* 1990;62:971–975.
17. Reedijk J, Lohman PHM. Cisplatin: synthesis, antitumour activity and mechanism of action. *Pharm Week Sci.* 1985;7:173–180.
18. Zunino F, Capranico G. DNA topoisomerase-II as the primary target of antitumor anthracyclines. *Anticancer Drug Des.* 1990;5:307–317.
19. Parker WB, Cheng YC. Metabolism and mechanism of action of 5-fluorouracil. *Pharmacol Ther.* 1990;48:381–395.
20. Wolff JE, Trilling T, Molenkamp G, Egeler RM, Jurgens H. Chemosensitivity of glioma cells in vitro: a meta analysis. *J Cancer Res Clin Oncol.* 1999;125: 481–486.
21. van Waarde A, Buursma AR, Hospers GA, et al. Tumor imaging with two sigma-receptor ligands, ^{18}F -FE-SA5845 and ^{11}C -SA4503: a feasibility study. *J Nucl Med.* 2004;45:1939–1945.
22. van Waarde A, Jager PL, Ishiwata K, Dierckx RA, Elsinga PH. Comparison of sigma-ligands and metabolic PET tracers for differentiating tumor from inflammation. *J Nucl Med.* 2006;47:150–154.
23. Kawamura K, Elsinga PH, Kobayashi T, et al. Synthesis and evaluation of ^{11}C - and ^{18}F -labeled 1-[2-(4-alkoxy-3-methoxyphenyl)ethyl]-4-(3-phenylpropyl)pi-perazines as sigma receptor ligands for positron emission tomography studies. *Nucl Med Biol.* 2003;30:273–284.
24. Schott B, Robert J. Comparative cytotoxicity, DNA synthesis inhibition and drug incorporation of eight anthracyclines in a model of doxorubicin-sensitive and -resistant rat glioblastoma cells. *Biochem Pharmacol.* 1989;38:167–172.
25. Mineura K, Kowada M. Enhancement of 5-fluorouracil cytotoxicity by cisplatin in brain tumour cell lines. *Cell Biol Int.* 1996;20:355–357.
26. Mares V, Giordano PA, Mazzini G, et al. Influence of cis-dichlorodiamine-platinum on glioma cell morphology and cell cycle kinetics in tissue culture. *Histochem J.* 1987;19:187–194.
27. Fornari FA Jr, Jarvis DW, Grant S, et al. Growth arrest and non-apoptotic cell death associated with the suppression of c-myc expression in MCF-7 breast tumor cells following acute exposure to doxorubicin. *Biochem Pharmacol.* 1996; 51:931–940.
28. Yoshikawa R, Kusunoki M, Yanagi H, et al. Dual antitumor effects of 5-fluorouracil on the cell cycle in colorectal carcinoma cells: a novel target mechanism concept for pharmacokinetic modulating chemotherapy. *Cancer Res.* 2001;61:1029–1037.
29. Davoodpour P, Bergstrom M, Landstrom M. Effects of 2-methoxyestradiol on proliferation, apoptosis and PET-tracer uptake in human prostate cancer cell aggregates. *Nucl Med Biol.* 2004;31:867–874.
30. Barthel H, Cleij MC, Collingridge DR, et al. 3'-Deoxy-3'-[^{18}F]fluorothymidine as a new marker for monitoring tumor response to antiproliferative therapy in vivo with positron emission tomography. *Cancer Res.* 2003;63:3791–3798.
31. Carnochan P, Brooks R. Radiolabelled 5-iodo-2'-deoxyuridine: a promising alternative to [F-18]-2-fluoro-2-deoxy-D-glucose for PET studies of early response to anticancer treatment. *Nucl Med Biol.* 1999;26:667–672.
32. Dittmann H, Dohmen BM, Kehlbach R, et al. Early changes in [F-18]FLT uptake after chemotherapy: an experimental study. *Eur J Nucl Med Mol Imaging.* 2002;29:1462–1469.
33. Fleischmannova V, Schmeer A, Drobnik J, Lodin Z, Mares V. The influence of drugs with a cytostatic action on the growth and differentiation of glioma cells in tissue culture [abstract]. *Physiol Bohemoslov.* 1982;31:441.
34. Higashi K, Clavo AC, Wahl RL. Does FDG uptake measure proliferative activity of human cancer cells? In vitro comparison with DNA flow cytometry and tritiated thymidine uptake. *J Nucl Med.* 1993;34:414–419.
35. Rasey JS, Grierson JR, Wiens LW, Kolb PD, Schwartz JL. Validation of FLT uptake as a measure of thymidine kinase-1 activity in A549 carcinoma cells. *J Nucl Med.* 2002;43:1210–1217.

36. Scanlon KJ, Safirstein RL, Thies H, Gross RB, Waxman S, Guttenplan JB. Inhibition of amino acid transport by cis-diamminedichloroplatinum(II) derivatives in L1210 murine leukemia cells. *Cancer Res.* 1983;43:4211–4215.
37. Mineura K, Sasajima T, Sasajima H, Kowada M. Inhibition of methionine uptake by cis-diamminedichloroplatinum (II) in experimental brain tumors. *Int J Cancer.* 1996;67:681–683.
38. Shirasaka T, Shimamoto Y, Ohshimo H, Saito H, Fukushima M. Metabolic basis of the synergistic antitumor activities of 5-fluorouracil and cisplatin in rodent tumor models in vivo. *Cancer Chemother Pharmacol.* 1993;32:167–172.
39. Toyohara J, Waki A, Takamatsu S, Yonekura Y, Magata Y, Fujibayashi Y. Basis of FLT as a cell proliferation marker: comparative uptake studies with [³H]thymidine and [³H]arabinothymidine, and cell-analysis in 22 asynchronously growing tumor cell lines. *Nucl Med Biol.* 2002;29:281–287.
40. Kong XB, Zhu QY, Vidal PM, et al. Comparisons of anti-human immunodeficiency virus activities, cellular transport, and plasma and intracellular pharmacokinetics of 3'-fluoro-3'-deoxythymidine and 3'-azido-3'-deoxythymidine. *Antimicrob Agents Chemother.* 1992;36:808–818.