Lack of Correlation Between Tritiated Deoxyglucose, Thallium-201 and Technetium-99m-MIBI Cell Incorporation Under Various Cell Stresses

Daniel O. Slosman and Jérôme Pugin

Nuclear Medicine Division, Geneva University Hospital, Geneva, Switzerland and the Scripps Research Institute, La Jolla, California

The use of fluorodeoxyglucose (FDG) and PET, recognized as an accurate tool for the specific diagnosis and staging of cancer, is currently being tested to monitor cancer therapy. Similar investigations have been performed with the nonPET markers ^{201}Tl and ^{99m}Tc- methoxyisobutylisonitrile (MIBI), two markers of myocardial perfusion shown to concentrate in malignant cells. We have tested the hypothesis that the cellular incorporation of ^{201}Tl and ^{99m}Tc-MIBI reflects that of FDG and correlates with treatment efficacy. **Methods:** We measured the incorporation in U937 cells of tritiated deoxyglucose (^{3}H-DG), ^{201}Tl and ^{99m}Tc-MIBI in basal conditions after stimulation or inhibition of the glucose metabolic pathway and after exposure to toxic agents selected to mimic the effects of chemotherapy. Thallium-201 or ^{99m}Tc-MIBI cell incorporation remained at basal levels after exposure to insulin, whereas ^{3}H-DG cell incorporation was greatly enhanced. Conversely, in the presence of 50 μM of NaF for 3 hr, only ^{3}H-DG cell incorporation was reduced to 57.2% ± 6.2% from control conditions. Cycloheximide (CYX), metaiodobenzylguanidine (MIBG) and bleomycin (BLM) were added to cell cultures. **Results:** Neither ^{201}Tl nor ^{99m}Tc-MIBI followed the changes in cell incorporation observed with ^{3}H-DG. In addition, only ^{3}H-DG cell incorporation was inversely correlated to the time of cell exposure or to the cell culture concentration of MIBG and BLM. **Conclusion:** In this model, cell incorporation of ^{201}Tl or ^{99m}Tc-MIBI differed from cell incorporation of ^{3}H-DG suggesting that it was not directly related to cell glycolysis activity and cell injury. In conclusion, these results do not support the hypothesis that ^{201}Tl or ^{99m}Tc-MIBI could replace FDG to monitor cancer treatment.

Key Words: fluorodeoxyglucose; positron emission tomography; cell toxicity; thallium-201; methoxyisobutylisonitrile

J Nucl Med 1994; 35:120–126

For more than a decade, ^{18}F-fluorodeoxyglucose has been the most frequently used marker for positron emission tomography (PET) imaging of malignant tumors, as it is recognized that the rate of glycolysis is very often increased in malignant cells (*1–3*). More recently, the use of FDG was proposed to monitor the efficacy of antitumoral treatment (*4–8*). Two nonPET markers widely used in cardiology to assess myocardial perfusion, ^{201}Tl and technetium-99m-labeled methoxyisobutylisonitrile (^{99m}Tc-MIBI), have been shown in the clinical setting to concentrate in tumors (*9–13*). Because preliminary results showed that ^{201}Tl or ^{99m}Tc-MIBI compared favorably to FDG imaging, they could represent potential alternative nonPET markers to the PET marker FDG for the diagnosis of tumors and the evaluation of treatment efficacy. Experimental data are still lacking to support the hypothesis that ^{201}Tl and ^{99m}Tc-MIBI reflect FDG cell incorporation. Therefore, we have compared in vitro the changes of cellular incorporation of tritiated deoxyglucose (^{3}H-DG) with those of ^{201}Tl and ^{99m}Tc-MIBI in the presence of various cellular poisons, including the antitumoral agent bleomycin (BLM).

MATERIAL AND METHODS

Determinations of cellular incorporation of ^{3}H-DG, ^{201}Tl and ^{99m}Tc-MIBI were conducted in parallel for the three markers, simultaneously with the determination of the number of viable cells by a colorimetric assay. In all cases, cellular incorporation of the markers was measured in steady-state conditions after exposure of the labeled compounds for 60 min.

Radiopharmaceuticals, Cells and Media

The radiopharmaceuticals 2-deoxy-D-1-^{3}H-glucose (13.9 Ci/mmole, Rahn Amersham, Zürich, Switzerland) and ^{201}Tl (Mallinckrodt Medical, Oryx Pharma, Zürich, Switzerland) were obtained from commercial sources, while ^{99m}Tc-MIBI was prepared from lyophilized kits (100 mCi/20 ml; Cardiolite, Heider, Wintherthur, Switzerland). The radiochemical purity of ^{99m}Tc-MIBI was verified using aluminum oxide TLC and ethanol according to the manufacturer's recommendations and ranged from 95% to 99%. A fast-growing human premonocytic line (U937, 12

Received Apr. 15, 1993; revision accepted Sept. 27, 1993.
For correspondence and reprints contact: Slosman Daniel, MD, Nuclear Medicine Division, Geneva University Hospital, 1211 Geneva 14. Switzerland.

hr of doubling time) was used for these experiments (*14*). The cells were maintained in stationary suspension in the medium RPMI-1640 supplemented with fetal calf serum (10%), glutamine (1%) and HEPES (25 m*M*).

Radiopharmaceutical Uptake Studies

First, 4×10^6 cells in 10 ml of medium were distributed in 3.5-cm dishes and exposed to specific agents and/or conditions during determined time intervals. The cells were then centrifuged, washed twice in PBS and resuspended in fresh medium at 10^5 cells/ml. Cell suspensions (200 μl) were distributed in each of the 96 wells of a microtiter plate. Either ^{3}H-DG (1 μCi/well), ^{201}Tl (1 μCi/well) or ^{99m}Tc-MIBI (50 μCi/well) were added for 60 min in specific wells. Then, cells were collected with an automated cell harvester on filter strips (Skatron type 11019, Tecnomara AG, Zürich, Switzerland), dried and counted with a gamma-counter (Cobra 5003, Canberra-Packard, Downers Grove, IL) (^{201}Tl and ^{99m}Tc-MIBI) or a beta-counter (Packard Tri-Carb, Canberra-Packard, Downers Grove, IL) in scintillation liquid (Ultima Gold, Canberra-Packard, Downers Grove, IL) (^{3}H-DG).

To assess the similarities in the cell incorporation mechanisms that may exist between ^{3}H-DG and ^{201}Tl or ^{99m}Tc-MIBI, effects of insulin were determined after exposure with 10 IU/ml for 3 hr. To test the participation of Na-K ATPase activity on ^{3}H-DG, ^{201}Tl and ^{99m}Tc-MIBI cell incorporation, KCl (40 μM) and ouabain (1 mM) were added to the medium for 90 min or 6 hr while NaF (50 μM) was added in the medium for 3 hr. To determine whether or not cell incorporation of the markers was different under different cell stresses, the toxic effects of increasing concentrations of CYX (10, 20, 50 and 75 μg), MIBG (10, 50 and 100 μM) and BLM (5, 10 and 50 μM) were individually tested after 90 min, 6 and 24 hr of cell exposure.

Each experiment was performed in quadruplicate and their means were calculated and considered as the results of one experiment. Additional dishes remained free of toxic agents and were performed with identical procedures. Results shown for each condition tested was obtained from six to eight repeated experiments. Results (%E) were expressed as percent of cpm measured in control experiments:

$$\%E_{meas} \text{ (tracer uptake/well)} = (100/\text{cpm control}) * \text{cpm experiment.}$$

Then, a correction for the viable cell number ($\%E_{cor}$) according to the cell viability assay (MTT, see below) was performed:

$$\%E_{cor} = (\%E_{meas} * 100)/\%MTT,$$

with %MTT measured simultaneously to the experimental conditions tested.

Viable Cell Number Determination by the Tetrazolium Salt Colorimetric Assay

The quantitative number of viable cells was determined using the colorimetric MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (*15*). MTT was added to each well for 4 hr at room temperature according to the manufacturer's protocol (no. M2128, Sigma products, 9470 Buchs, Switzerland). Optical density of the microtiter plates was measured on a multiwell scanning spectrophotometer at 550 nm (Anthos Labtec Instruments HT 2, Kontron Instruments AG, 8952 Schlieren, Switzerland). Results were expressed as percent of the values obtained in the control experiments and were used to proportionally normalize $\%E_{meas}$.

Statistics

Results are presented as mean ± standard error of the mean (x ± s.e.m.) unless otherwise indicated. Multiple regression analysis was used to assess the influence of time and dose on measurement of the different markers tested. To test the significance of differences between measurements determined at a specific time of exposure with different doses, a one-way analysis of variance (ANOVA) and Bonferroni correction were used to determine the level of significance between the parameters tested.

RESULTS

After 90 min exposure to KCl, the viable cell number was not significantly influenced while in the presence of ouabain, %MTT was reduced to 89.3% ± 1.9% from control conditions. After 3 hr exposure to insulin or NaF, %MTT remained unchanged while after 6 hr of exposure to ouabain, %MTT was reduced to 74.4% ± 5.4% from control (Fig. 1). The cell incorporation of ^{3}H-DG corrected for viable cell number only was significantly increased by insulin: $\%E_{cor}$ = 162.7% ± 30.3% from control after exposure to 10 IU/ml insulin for 3 hr (Fig. 1). After addition of 50 μM of NaF in the medium for 3 hr, $\%E_{cor}$ ^{3}H-DG was decreased to 57.3% ± 6.3% from control (Fig. 1). The presence of KCl significantly reduced $\%E_{cor}$ ^{201}Tl but increased in the presence of ouabain (94.0% ± 1.8% and 138.6% ± 9.3%, respectively) while $\%E_{cor}$ ^{99m}Tc-MIBI was significantly increased in the presence of KCl (130.8% ± 12.8%) (Fig. 1).

The viable cell number was significantly reduced by the toxic compounds BLM, CYX and MIBG. When cells were exposed to BLM or CYX, %MTT was reduced as a function of the dose of the cytotoxic agent administered, as well as a function of the time of exposure (two-way ANOVA: $n = 54$, $p < 0.0001$ and $n = 81$, $p < 0.0005$, respectively). When cells were exposed to MIBG, the viable cell number was reduced as a function of the dose of the cytotoxic agent administered (two-way ANOVA, $n = 54$, partial F-value = 9.274, $p < 0.0005$) whereas the time of exposure of MIBG had a slight but significant effect (partial F-value = 2.867, $p < 0.05$). After 24 hr of exposure of the cells to the toxic compounds, the viable cell number was more affected by CYX (Table 1) than by BLM (Table 2) or MIBG (Table 3).

Changes in cell incorporation of ^{3}H-DG, ^{201}Tl and ^{99m}Tc-MIBI corrected for viable cell number were different according to the toxic compounds tested. When cells were exposed to CYX, $\%E_{cor}$ of all three markers used was increased significantly as a function of concentration or exposure time (Table 1). When cells were exposed to BLM, a significant decrease of $\%E_{cor}$ ^{3}H-DG was observed only with the largest dose (50 μM) and after 24 hr exposure ($\%E_{cor}$ = 60.4% ± 5.4%) (Table 2); conversely, $\%E_{cor}$ ^{201}Tl and ^{99m}Tc-MIBI was significantly increased as a function of dose (two-way ANOVA: $p < 0.05$ and $p < 0.0001$, respectively), but not time of exposure (Table 2). When cells were exposed to MIBG, $\%E_{cor}$ ^{201}Tl and ^{99m}Tc-MIBI

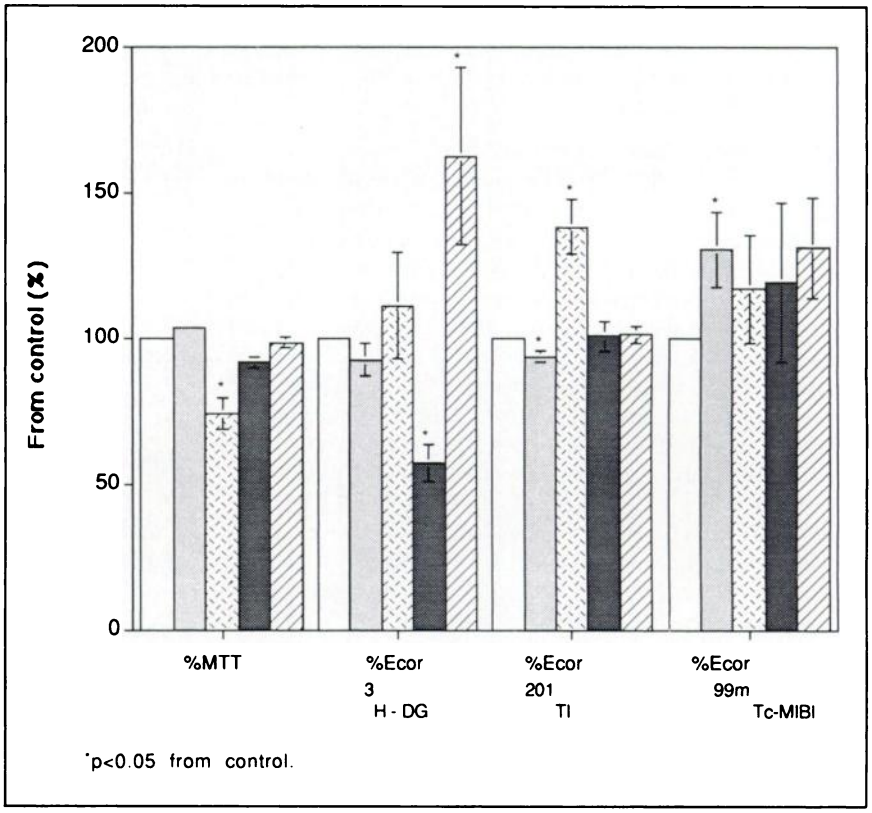

FIGURE 1. Measurements of viable cell number (%MTT) and cell incorporation (%E_{cor}) of ^{3}H-deoxyglucose (^{3}H-DG), ^{201}Tl and ^{99m}Tc-MIBI when cells were exposed to KCl (▨), ouabain (▨), NaF (■) or insulin (▨). Results (mean ± s.e.m.) are expressed as percent of the values measured in the control experiments (□).

never decreased whereas %E_{cor} ^{3}H-DG only significantly decreased when exposed to 100 μM of MIBG (Table 3).

As shown in Figure 2A, when %E_{meas} (tracer uptake/well) was considered after 24-hr exposure to CYX, %E_{meas} ^{3}H-DG appeared only to be significantly increased with 10 μg (178.6% ± 32.0%), while %E_{meas} ^{201}Tl was slightly but significantly increased at all doses and ^{99m}Tc-MIBI remained unchanged. After a 24-hr exposure to BLM, %E_{meas} ^{3}H-DG remained significantly decreased with 50 μM BLM (37.4% ± 2.7%), while %E_{meas} ^{201}Tl and ^{99m}Tc-

TABLE 1
Cell Incorporation of ^{201}Tl, ^{99m}Tc-MIBI and ^{3}H-DG Corrected for Viable Cell Number (%E_{cor}) When Cells Were Exposed to CYX*

Toxic agents	%E_{cor} (^{3}H-DG)	%E_{cor} (^{201}Tl)	%E_{cor} (^{99m}Tc-MIBI)
CYX (μg) for 90 min of exposure			
10	152.3 ± 16.5	113.1 ± 2.0	157.5 ± 13.1†
20	141.0 ± 76.6	106.3 ± 6.0	138.2 ± 19.8
50	146.5 ± 85.3	118.8 ± 22.3†	135.7 ± 48.8
75	174.6 ± 69.8†	118.4 ± 11.3†	148.0 ± 65.4†
CYX (μg) for 6 hr of exposure			
10	334.2 ± 51.0†	122.1 ± 22.4	189.6 ± 30.7†
20	299.4 ± 91.2†	143.9 ± 20.6†	183.9 ± 23.5†
50	185.2 ± 18.1	140.9 ± 10.0†	175.1 ± 28.2†
75	269.0 ± 54.6†	166.9 ± 16.0†	201.7 ± 30.2†
CYX (μg) for 24 hr of exposure			
10	276.7 ± 60.4	188.4 ± 11.4†‡	155.3 ± 4.9‡
20	241.3 ± 53.5	202.4 ± 10.0†‡	181.6 ± 5.5†‡
50	197.9 ± 50.7	222.4 ± 27.6†‡	201.5 ± 32.9†‡
75	281.9 ± 85.8†	302.8 ± 24.2†	308.6 ± 38.9†

*Results (mean ± s.e.m.) are expressed as percent of the values measured in the control experiments.
†$p < 0.05$ from control.
‡$p < 0.05$ from a 75-μg dose.

TABLE 2
Cell Incorporation of ^{201}Tl, ^{99m}Tc-MIBI and ^{3}H-DG Corrected for Viable Cell Number (%E_{cor}) When Cells Were Exposed to BLM*

Toxic agents	%E_{cor} (^{3}H-DG)	%E_{cor} (^{201}Tl)	%E_{cor} (^{99m}Tc-MIBI)
BLM (μM) for 90 min of exposure			
5	120.0 ± 19.8	117.2 ± 6.5	140.2 ± 11.0
10	118.8 ± 18.6	122.3 ± 7.9	154.2 ± 13.2
50	65.7 ± 18.7	107.9 ± 3.3	115.6 ± 6.4
BLM (μM) for 6 hr of exposure			
5	148.2 ± 23.4	122.9 ± 3.3†	122.3 ± 5.3†
10	210.4 ± 47.3	123.2 ± 6.2†	121.4 ± 3.6†
50	139.2 ± 42.6	119.3 ± 7.2	140.2 ± 5.3†‡
BLM (μM) for 24 hr of exposure			
5	91.4 ± 11.4	142.4 ± 18.8	189.0 ± 52.6†
10	113.7 ± 10.5	173.4 ± 20.8	217.1 ± 58.2†
50	60.4 ± 5.4†‡	195.3 ± 24.7†	229.9 ± 44.7†

*Results (mean ± s.e.m.) are expressed as percent of the values measured in the control experiments.
†$p < 0.05$ from control.
‡$p < 0.05$ from a 10-μM dose.

MIBI tended to increase at all doses but reached significance only at a dose of 10 μM with %E_{meas} ^{201}Tl (133.8% ± 11.3%) (Fig. 2B). After a 24-hr exposure to MIBG, %E_{meas} ^{3}H-DG remained significantly increased with 50 μM MIBG (153.8% ± 22.3%) and tended to decreased at a dose of 100 μM (61.9% ± 10.4%), while %E_{meas} ^{201}Tl was only slightly increased at all doses and ^{99m}Tc-MIBI remained unchanged (Fig. 2C).

As shown in Figure 3, linear regression analysis demonstrated the presence of a weak but significant correlation between %E_{cor} ^{3}H-DG and %E_{cor} ^{201}Tl ($n = 204$, $R = 0.345$, $p < 0.0001$) or %E_{cor} ^{99m}Tc-MIBI ($R = 0.299$, $p < 0.0001$) while %E_{cor} ^{201}Tl ($n = 204$, $R = 0.345$, $p < 0.0001$) and %E_{cor} ^{99m}Tc-MIBI appeared well correlated ($R = 0.673$, $p < 0.0001$). No significant correlation between ^{3}H-DG and ^{201}Tl or ^{99m}Tc-MIBI was found when %E_{meas} values were used.

DISCUSSION

Fluorine-18-FDG is a positron-emitting radiopharmaceutical used for more than a decade to diagnose cancer. The increased cell incorporation of FDG has been related to the increased metabolic activity of malignant cells. The mechanisms involved may be due, at least in part, to increased glucose transport through the cell membrane

TABLE 3
Cell Incorporation of ^{201}Tl, ^{99m}Tc-MIBI and ^{3}H-DG Corrected for Viable Cell Number (%E_{cor}) When Cells Were Exposed to MIBG*

Toxic agents	%E_{cor} (^{3}H-DG)	%E_{cor} (^{201}Tl)	%E_{cor} (^{99m}Tc-MIBI)
MIBG (μM) for 90 min of exposure			
10	94.9 ± 11.3	98.2 ± 2.7	110.5 ± 3.4
50	109.6 ± 21.3	101.1 ± 7.4	126.7 ± 9.6
100	79.9 ± 13.1	103.0 ± 5.7	172.6 ± 48.7
MIBG (μM) for 6 hr of exposure			
10	130.6 ± 15.6	130.2 ± 13.1	128.3 ± 10.9
50	138.1 ± 17.5	113.5 ± 6.5	133.1 ± 12.4
100	89.3 ± 14.6	99.7 ± 13.9	142.6 ± 30.9
MIBG (μM) for 24 hr of exposure			
10	100.4 ± 14.2	111.0 ± 6.1	100.7 ± 6.6
50	159.8 ± 25.6	117.5 ± 8.1	114.1 ± 7.3
100	77.2 ± 14.3§	128.6 ± 6.6†	157.5 ± 21.7†‡

*Results (mean ± s.e.m.) are expressed as percent of the values measured in the control experiments.
†$p < 0.05$ from control.
‡$p < 0.05$ from a 10-μM dose.
§$p < 0.05$ from a 50-μM dose.

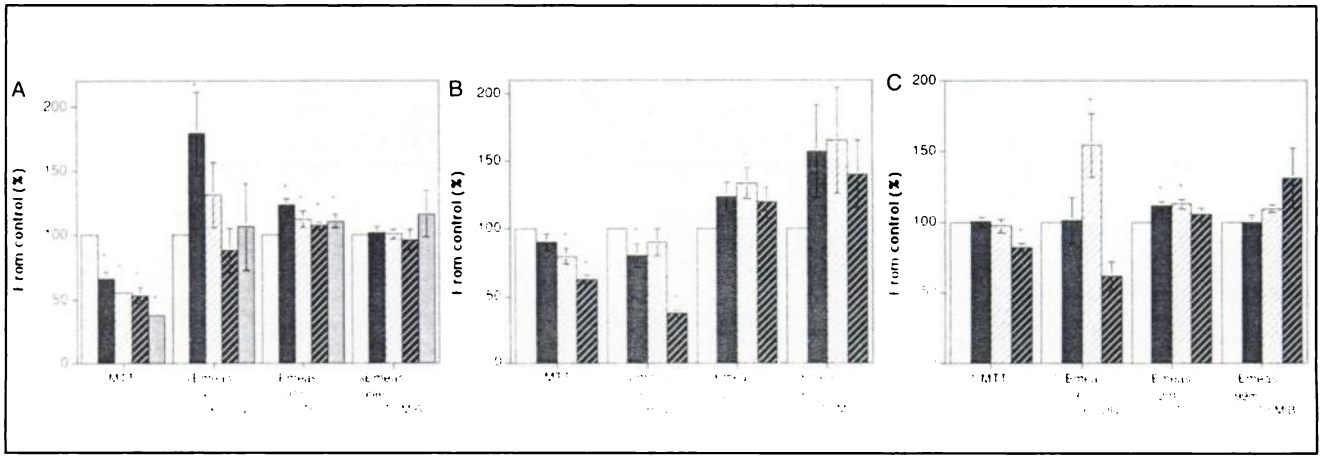

FIGURE 2. (A) Measurements of viable cell number (%MTT) and cellular incorporation (%E_{meas}) of ^{3}H-deoxyglucose (^{3}H-DG), ^{201}Tl and ^{99m}Tc-MIBI when cells were exposed to cycloheximide (CYX): 10 μM (■), 20 μM (▨), 50 μM (■) and 75 μM (▨). Results (mean ± s.e.m.) are expressed as percent of the values measured in the control experiments (□). (B) Measurements of viable cell number (%MTT) and cellular incorporation (%E_{meas}) of ^{3}H-deoxyglucose (^{3}H-DG), ^{201}Tl and ^{99m}Tc-MIBI when cells were exposed to bleomycin (BLM): 5 μM (■), 10 μM (▨) and 50 μM (■). Results (mean ± s.e.m.) are expressed as percent of the values measured in the control experiments (□). (C) Measurements of viable cell number (%MTT) and cellular incorporation (%E_{meas}) of ^{3}H-deoxyglucose (^{3}H-DG), ^{201}Tl and ^{99m}Tc-MIBI when cells were exposed to metaiodobenzylguanidine (MIBG): 10 μM (■), 50 μM (▨) and 100 μM (■). Results (mean ± s.e.m.) are expressed as percent of the values measured in the control experiments (□).

and/or to increased enzymatic glycolytic activity or, more generally, to increased metabolism of recovery.

Numerous clinical observations have shown that ^{201}Tl could also be used to visualize malignancy and recent reports suggest that it may be an indicator as reliable as FDG for cancer detection using SPECT. Technetium-99m-MIBI has also been proposed for cancer imaging because of its similarity of behavior to ^{201}Tl (*9–13*). At the cellular level, the increased incorporation of ^{201}Tl has been suggested to be due to an increased intracellular transport similar to the sodium ATP-dependent transport of potassium. Plasma and mitochondrial membrane potentials appear to play an important role for ^{99m}Tc-MIBI (*16*).

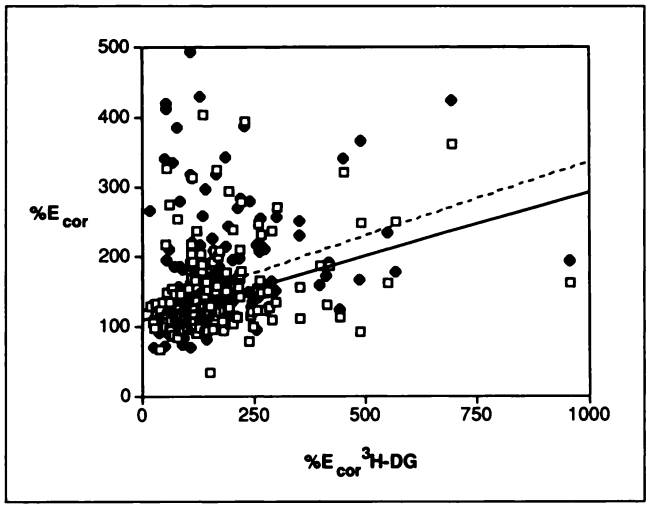

FIGURE 3. Correlation between cellular incorporation (%E_{cor}) of ^{3}H-deoxyglucose (^{3}H-DG) and ^{201}Tl (□) or ^{99m}Tc-MIBI (◆). Linear regressions are shown for ^{201}Tl (dotted line) and for ^{99m}Tc-MIBI (plain line).

As little is known about the behavior of these markers during therapy, this in vitro study was designed to investigate study modifications of cellular incorporation of ^{201}Tl and ^{99m}Tc-MIBI compared with tritiated deoxyglucose when cells are exposed to toxic agents. In our experiments, we were measuring the accumulation of markers that represented the net result of both influx and washout. This has the advantage of reflecting the amount of marker in the tissue at the end of the scan, but it has the disadvantage of concealing the specific effects of each component.

We observed that deoxyglucose incorporation in tumor cells differs from that of ^{201}Tl or ^{99m}Tc-MIBI in the first set of experiments (insulin, KCl, NaF, ouabain). Cell incorporation of glucose is a complex procedure that may vary depending on the type of cells studied (*17*) and on their insulin-sensitivity (*18*). In our model, we found that exposure to insulin enhanced DG incorporation but not ^{201}Tl or ^{99m}Tc-MIBI. Furthermore, the addition of NaF to the culture medium has a unique inhibitory effect on ^{3}H-DG cell incorporation. Therefore, U937 cells are insulin-sensitive and their glucose transport is most likely regulated by glucose transporters and phosphorylation activity (*18*).

We also observed that ^{201}Tl cell incorporation could only be slightly reduced by the presence of an overload of KCl in the medium while neither KCl nor ouabain depressed ^{99m}Tc-MIBI. On the contrary, no effects were observed for ^{3}H-DG cell incorporation. The main factors described to control muscle cell incorporation of ^{201}Tl are the Na^{+}-K^{+} ATPase system (*19,20*), pH and oxygenation (*21*). In squamous cell carcinoma in culture, Schweil et al. found some evidence that the Na^{+}-K^{+} ATPase system was also involved (*22*). Our results are also supported by the work of Krivokapich and Shine in which they concluded that the sodium-potassium pump did not participate in the influx of

tracer amounts of ^{201}Tl but participated, to a certain extent, in the net cell accumulation of ^{201}Tl (*23*). Regarding ^{99m}Tc-MIBI cell incorporation, Delmon-Moingeon et al. showed that it might vary from 5% to 28% depending upon the tumor cell studied and that it could be depressed by hyperkaliema (*16*). They also demonstrated that in chick myocardial cells, plateau levels of ^{99m}Tc-MIBI were increased in the presence of ouabain (*24*). These results are in agreement with our observations. Therefore, looking at the net cellular accumulation of the markers in the specific conditions tested (insulin, KCl, NaF, ouabain), ^{3}H-DG cell incorporation differs from that of ^{201}Tl or ^{99m}Tc-MIBI, as expected.

The behavior of deoxyglucose incorporation in tumor cells also differs from that of ^{201}Tl or ^{99m}Tc-MIBI when cell-suffering is induced by a toxic agent. The toxic mechanism of cycloheximide, MIBG and bleomycin are clearly demonstrated by the significant reduction in the number of viable cells as a function of time and dosage of the compounds. Cell incorporation of ^{3}H-DG, ^{201}Tl and ^{99m}Tc-MIBI is directly related to the effects of the toxic compounds. Higashi and Wahl showed that FDG cell uptake is correlated with the number of viable cells in cell culture under normal conditions (*25*), but the authors did not investigate what the relationship under cell stress would be. Transient increased anaerobic glycolysis could be the major mechanism by which the cells attempt to remain metabolically active and this would explain the transient increase in $\%E_{cor}$ of ^{3}H-DG at intermediate concentrations of MIBG, whereas at high concentrations, the cells were no longer able to maintain normal metabolic function and the remaining viable cells showed decreased $\%E_{cor}$ ^{3}H-DG. It should be noted that cell incorporation of ^{201}Tl and ^{99m}Tc-MIBI is increased simultaneously with the reduction of $\%E_{cor}$ ^{3}H-DG. The toxic mechanism by which BLM acts on the cells does not involve alterations of enzymatic activity but directly involves DNA by multiple-strand scission. The toxicity of BLM was clearly demonstrated by the reduction in cell mass by the MTT assay and $\%E_{cor}$ was decreased for the three markers. This led us to suggest that BLM may also act on the mitochondrial DNA and therefore indirectly alter mitochondrial function. The last toxic compound, CYX, alters protein synthesis and is a multitarget agent that does not directly act on mitochondrial function so cells are most likely attempting to survive by increasing anaerobic glycolysis. Our data extend the previous observations made by Haberkorn et al. (*26*); U937 cells exposed to stress or injury, that suffered and showed a depressed aerobic metabolism, responded to a certain extent by an increase in glucose incorporation due to stimulation of the anaerobic pathway.

Regarding ^{201}Tl and ^{99m}Tc-MIBI cell incorporation, discrepant behavior was observed. Based on the hypothesis that the Na-K ATPase pump participates in cell accumulation of ^{201}Tl in the presence of a specific toxic agent, a depressed cell incorporation of ^{201}Tl could be explained by a targeting of the Na-K ATPase pump. The increased incorporation observed in our experiments could not be explained on this basis. As mentioned earlier, ^{201}Tl has a complex mechanism of accumulation in cells. It involves the Na-K ATPase pump, specific ion channels and regulating the rates of transport through potassium concentration as described in the model published by Krivokapich and Shine (*23*).

Tumor cells may have additional mechanisms. The participation of increased membrane permeability and an associated increased diffusion could explain both the clinical observations and our results. The ^{99m}Tc-MIBI incorporation in tumor cells is related to the plasma and mitochondrial membrane potentials (*16*). Therefore, in the presence of cell injury where membrane alterations are present, modification of Ca^{++}-channel or Na-K ATPase pump functions might result in an increased cell uptake of ^{99m}Tc-MIBI as observed in the case of chick myocardial cells exposed to verapamil or ouabain (*24*). When cells were exposed to the toxic agent BLM, MIBG or CYX, the largest increase in cell incorporation was observed with ^{99m}Tc-MIBI. The participation of the calcium channel in the increase of ^{99m}Tc-MIBI cell incorporation may also be possible. Crane et al. observed that more than 90% of the marker was localized within the mitochondria and that its movement was dependent on the mitochondrial calcium channel (*27*). In our in vitro model, we attempt to mimic the in vivo effects of chemotherapy. The dose-dependent and time-dependent decreased %MTT attaining more than 50% after 24 hr of exposure with CYX and nearly 50% with BLM validate our model. Furthermore, by means of the limiting dilution analysis technique, we have already shown that cell proliferation was nearly halted with a similar BLM dosage (*28,29*).

For the purpose of imaging, the in vitro study of the tracer uptake normalized by the number of viable cells is not always an ideal model because the in vivo tumor contained damaged cells as well as necrosis, in addition to viable cells. Furthermore, the in vitro situation differs from in vivo systems in particular because of the absence of blood supply and infiltrating inflammatory cells. In an attempt to reflect the in vivo state, we also analyzed the data in terms of $\%E_{meas}$ (percentage of tracer uptake per well), therefore including the tracer content of the dead cells. With this method of analysis, we observed identical trends: increased $\%E_{meas}$ ^{3}H-DG, ^{201}Tl and ^{99m}Tc-MIBI when cells were exposed to CYX, BLM or MIBG. The only differences between $\%E_{meas}$ and $\%E_{cor}$ were observed when cells were exposed to the largest doses of the toxic compounds tested; a decrease in $\%E_{meas}$ while $\%E_{cor}$ remained elevated. This discrepancy reflects most likely a major loss of cells and an imbalance in the ratio between the increased metabolic activity of the survival cells and the limited tracer content of the dead cells.

In conclusion, using U937 cells, we made in vitro experimental observations to demonstrate that deoxyglucose incorporation behavior in these cells differs from those of ^{201}Tl or ^{99m}Tc-MIBI when cell-suffering is induced by toxic

agents. Therefore, we may conclude that there is no direct experimental evidence to support the hypothesis that ^{201}Tl or ^{99m}Tc-MIBI can predict cancer therapy efficacy. In addition, transiently-increased uptake of FDG may be encountered in the presence of cell suffering when anaerobic glycolysis is activated and this may lead to false conclusions of quantitative PET scan.

ACKNOWLEDGMENTS

The authors thank Nicole Pittet and Laurence Porret for their technical assistance. This work was supported by the Swiss National Science Foundation (Grant no. 32-028645.90).

REFERENCES

1. Yonekura Y, Benua RS, Brill AB, Som P, Atkins HL. Increased accumulation of 2-deoxy-2[^{18}F]fluoro-D-glucose in liver metastases from colon cancer. *J Nucl Med* 1982;23:1133–1137.
2. Di Chiro G, Hatazawa J, Katz DA, Rizzoli HV, De Michele DJ. Glucose utilization by intracranial meningiomas as an index of tumor aggressivity and probability of recurrence: a PET study. *Radiology* 1987;164:521–526.
3. Strauss LG, Conti PS. The applications of PET in clinical oncology. *J Nucl Med* 1991;32:623–648.
4. Iosilevsky G, Front D, Bettman L, Hardoff R, Ben-Arieh Y. 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.
5. Kubota K, Ishiwata K, Kubota R, et al. Tracer feasibility for monitoring tumor radiotherapy: a quadruple tracer study with fluorine-18-fluorodeoxyglucose or fluorine-18-fluorodeoxyuridine, L-(methyl-^{14}C)methionine, (6-^{3}H)thymidine, and gallium-67. *J Nucl Med* 1991;32:2118–2123.
6. Okada J, Yoshikawa K, Imazeki K, et al. The use of FDG-PET in the detection and management of malignant lymphoma: correlation of uptake with prognosis. *J Nucl Med* 1991;32:686–691.
7. Okazumi S, Isono K, Enomoto K, et al. Evaluation of liver tumors using fluorine-18-fluorodeoxyglucose PET: characterization of tumor and assessment of effect of treatment. *J Nucl Med* 1992;33:333–339.
8. Haberkorn U, Strauss LG, Dimitrakopoulou AD, et al. Fluorodeoxyglucose imaging of advanced head and neck cancer after chemotherapy. *J Nucl Med* 1993;34:12–17.
9. Kaplan WD, Takvorian T, Morris JH, Rumbaugh CL, Connoly BT, Atkins HL. Thallium-201 brain tumor imaging: a comparative study with pathologic correlation. *J Nucl Med* 1987;28:47–52.
10. Schwartz RB, Carvahlo PA, Alexander III E, Loeffler JS, Folkerth R, Holman BL. Radiation necrosis versus high-grade recurrent glioma: differentiation by using dual-isotope SPECT with ^{201}Tl and ^{99m}Tc-HMPAO. *AJNR* 1992;12:1187–1192.
11. Matsuno S, Tanabe M, Kawasaki Y, et al. Effectiveness of planar image and single photon emission tomography of thallium-201 compared with gallium-67 in patients with primary lung cancer. *Eur J Nucl Med* 1992;19:86–95.
12. Caner B, Kitapçl M, Unlü M, et al. Technetium-99m-MIBI uptake in benign and malignant bone lesions: a comparative study with technetium-99m-MDP. *J Nucl Med* 1992;33:319–324.
13. Bagni B, Tamarozzi R, Calzolari F, Ceruti S. SPECT imaging of brain tumors with Tc-99m MIBI. *Radiology* 1992;185(P):A769;233.
14. Sundström C, Nilsson K. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). *Int J Cancer* 1976;17:565–577.
15. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983;65:55–63.
16. Delmon-Moingeon LI, Piwnica-Worms D, Van den Abbeele AD, Holman BL, Davison A, Jones AG. Uptake of the cation hexakis(2-methoxyisobutylisonirile)-technetium-99m by human carcinoma cell lines in vitro. *Cancer Res* 1990;50:2198–2202.
17. Widdas WF. Old and new concepts of the membrane transport for glucose in cells. *Biochemica et Biophysica Acta* 1988;947:385–404.
18. Joost HG, Weber TM. The regulation of glucose transport in insulin-sensitive cells. *Diabetologica* 1989;32:831–838.
19. Gehring PJ, Hammond PB. The interrelationship between thallium and potassium in animals. *J Pharmacol Exp Ther* 1967;155:187–201.
20. Pohost GM, Alpert NM, Ingwall JS, Strauss WH. Thallium redistribution: mechanisms and clinical utility. *Semin Nucl Med* 1980;10:70–93.
21. Weich HF, Strauss WH, Pitt B. The extraction of thallium-201 by the myocardium. *Circulation* 1977;56:188–191.
22. Schweil AM, McKillop JH, Milroy R, Wilson R, Abdel-Dayem HM, Omar YT. Mechanism of ^{201}Tl uptake in tumors. *Eur J Nucl Med* 1989;15:376–379.
23. Krivokapich J, Shine KI. Effects of hyperkalemia and glycoside on thallium exchange in rabbit ventricle. *Am J Physiol* 1981;240:H612–H619.
24. Piwnica-Worms D, Kronauge JF, Delmon L, Holman BL, Marsh JD, Jones AG. Effect of metabolic inhibition on technetium-99m-MIBI kinetics in cultured chick myocardial cells. *J Nucl Med* 1990;31:464–472.
25. Higashi K, Wahl RL. In vitro comparison of FDG, methionine and thymidine as possible PET tumor imaging agents. *Radiology* 1992;185(P):A49; 109.
26. Haberkorn U, Reinhardt M, Strauss LG, et al. Metabolic design of combination therapy: use of enhanced fluorodeoxyglucose uptake caused by chemotherapy. *J Nucl Med* 1992;33:1981–1987.
27. Crane P, Laliberté R, Heminway S, Thoolen M, Orlandi C. Effect of mitochondrial viability and metabolism on technetium-99m-sestamibi myocardial retention. *Eur J Nucl Med* 1993;20:20–25.
28. Slosman DO, Pitet N, Polla BS. In vitro comparison of cytotoxic and antiproliferative effects of cold and ^{131}I-labeled MIBG. *Eur J Nucl Med* 1992;19:A241:607.
29. Slosman DO, Pittet N, Donath A, Polla BS. Deoxyglucose cell incorporation as an index of cell proliferation. *Eur J Nucl Med* 1993;20:1084–1088.