Effect of γ-radiation on the ratio of [^{18}F]2-fluoro-2-deoxy-D-glucose to glucose utilization in human glioblastoma cells *in vitro*

F H A SCHNEEWEISS, R N SHARAN*,†, H HAUTZEL**, H W MÜLLER-GÄRTNER**

Institute of Medicine, Research Center Jülich, 52425 Jülich, Germany

**Radiation and Molecular Biology Unit, Department of Biochemistry, North-Eastern Hill University, Umshing, Shillong 793 022, India*

***Clinic of Nuclear Medicine, Heinrich-Heine-University of Düsseldorf, 40225 Düsseldorf, Germany*

†Corresponding author (Fax, 91-364-250108).

The glucose consumption in tumours *in vivo* as reflected by uptake of [^{18}F]2-fluoro-2-deoxy-D-glucose (^{18}FDG) using positron emission tomography (PET) is currently under investigation as a measure of tumour response to radiotherapy. The calculation of cerebral metabolic rate of glucose from ^{18}FDG-PET data requires a proportionality factor referred to as the lumped constant. In the present *in vitro* study, the utilizations of ^{18}FDG and glucose have been measured in a human glioblastoma cell line (86HG-39) as a function of γ-radiation dose with various post-irradiation times and of different fractionation modes. The ratio of utilization of ^{18}FDG to that of glucose ($R_{F/G}$), assumed to correspond to the lumped constant, was observed to increase 12 and 24 h after single fraction γ-exposure by factors ranging from 1·2 to 1·5 compared with the non-irradiated controls. It decreased after multiple fraction γ-exposure (4×2 Gy) by a factor of 0·7 compared with the single fraction schedule (1×8 Gy). The results suggest that the affinities of glucose transporters or hexokinase enzyme or both for ^{18}FDG and glucose could be influenced by γ-irradiation in this tumour cell line *in vitro*. Apparent changes of the glucose consumption determined with PET in human tumours following radiotherapy may, therefore, not be solely due to changes in cellular metabolism or cell number but may also be due to changes in $R_{F/G}$.

1. Introduction

An increased energy demand is a well recognized characteristic of most malignant tumours. Glucose is the major source of energy to living cells and cancerous cells are known to show enhanced consumption of glucose signifying the increased energy demand. The advent of novel *in vivo* imaging methods, such as positron emission tomography (PET), and the concomitant development of appropriate mathematical models (Sokoloff *et al* 1977) have enabled the determination of the glucose metabolic rate for the human brain. The *in vivo* calculation of tumour glucose metabolic rate by PET using [^{18}F]2-fluoro-2-deoxy-D-glucose (^{18}FDG) may be of value in predicting the clinical response of tumours to radiotherapy and chemotherapy.

The model which has been developed by Sokoloff *et al* (1977) for *in vivo* quantification of cerebral glucose metabolic rate in rats is based upon the assumption that the utilization of FDG during a limited time of less than 1 h is proportional to that of glucose. For normal human subjects Lucignani *et al* (1993) have found the optimal time between 60 and 120 min. No conclusive estimate is available for tumours. For an *in vitro* model, where equilibration is attained faster than a complex *in vivo* system, a 45 min period for irreversible entrapment of deoxyglucose-6-phosphate in cells may be reasonable (Sokoloff *et al* 1977; Herholz *et al* 1990; Som *et al* 1980). It is further assumed that the utilization of glucose and ^{18}FDG reach and maintain steady state during this period (Sokoloff *et al* 1977). This was experimentally verified *in vivo* (Reivich *et al* 1985). The lumped constant

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(LC) in Sokoloff's model represents the relation between the kinetics of ^{18}FDG to those of glucose (Herholz *et al* 1990; Gjedde *et al* 1985). Since the LC is reported to change under pathological conditions (Gjedde *et al* 1985), it may also be affected by therapeutic interventions, such as radiotherapy or chemotherapy. The *in vivo* measurement of changes of the LC after therapeutic interventions is rather difficult. In this study, an *in vitro* model for determination of changes of the ratio between ^{18}FDG- and glucose utilizations ($R_{F/G}$) under steady state conditions has been developed assuming that this ratio corresponds to the LC *in vivo*. Using the *in vitro* model, radiation induced changes on the $R_{F/G}$ has been measured. The *in vitro* study determines the $R_{F/G}$ as a function of the γ-radiation dose at various post-irradiation times and of different modes of irradiation in monolayers of a human glioblastoma cell line.

2. Materials and methods

2.1 *Cell culture*

Human glioblastoma (HG) cells, 86HG-39, were cultured as monolayer in Leighton tubes (NUNC, Denmark). Approximately $0{\cdot}3 \times 10^6$ cells were seeded in each Leighton tube and were grown to confluence for 6 days at 37°C and in the presence of 5% CO_2 in 3 ml Iscove's modified Dulbecco's medium, 10% fetal calf serum (FCS, Gibco BRL, England), and 1% penicillin/streptomycin. After removal of the growth medium, each monolayer ($2{\cdot}3 \times 10^6$ cells) was washed with 3 ml of Dulbecco's minimum essential medium (DMEM, Biochrom, Germany) containing 10% FCS and incubated in 3 ml of fresh DMEM for 1 h. During this time the cells which had grown in the presence of 23 mM glucose were equilibrated to 6 mM glucose of DMEM. Under these experimental conditions, the majority of HG cells were in G_0/G_1-phase of the cell cycle (G_0/G_1 : 79·3 ± 3·3%, G_2 : 9·8 ± 2·4%, S : 11·2 ± 1·7%). The doubling time of this cell line was about 36 h.

2.2 *Calculation of $R_{F/G}$*

For the measurement of ^{18}FDG uptake the equilibration medium, DMEM, was replaced by 1 ml of fresh DMEM containing 10% FCS, 6 mM glucose, and 7·4 kBq (0·2 µCi) and ^{18}FDG. ^{18}FDG was produced at the Institute of Nuclear Chemistry of the Research Center Jülich (Hamacher *et al* 1986). The radioactivity of the medium of each sample was measured with a gamma-counter (Gammaszint model 5300, Berthold and Friesecke) at the beginning and at the end of 45 min incubation at 37°C with mild shaking (20 cycles min^{-1}). The difference of the two measurements after the correction of ^{18}F-decay was the loss of ^{18}FDG from the extracellular medium representing the utilization of ^{18}FDG by 86HG-39 cells. The loss of extracellular glucose, monitored by hexokinase and 6-phosphate dehydrogenase assay using the auto-analyzer RA-X (Technicon, Bayer), represented glucose utilization. The $R_{F/G}$ was calculated using these two measurements.

2.3 *Exposure of cells to γ-radiation*

A ^{137}Cs-source (Gammacell 40, Atomic Energy of Canada Ltd.) was used for γ-irradiation (dose rate: 1·07 Gy min^{-1}).

2.3a *Single fraction exposure to γ-radiation*: Leighton tubes containing monolayers of $2{\cdot}3 \times 10^6$ human glioblastoma cells were irradiated at room temperature with 1, 2, 8 or 16 Gy. The $R_{F/G}$ was determined 4, 12 or 24 h after termination of the γ-irradiation. The post-irradiation incubation was carried out at 37°C.

2.3b *Single fraction versus multiple fractions exposure to γ-radiation*: Leighton tubes with cell monolayers (see above) were irradiated at room temperature four times with 2 Gy each (multiple fraction). After each fraction of irradiation the tubes were left at 37°C for 12 h. These were compared with samples which were irradiated with 8 Gy γ-rays (single fraction) and left for 12 h at 37°C after irradiation.

Data points represent at least 5 independent experiments each comprising 3–5 replicates. The controls were sham-irradiated and kept under identical conditions as the irradiated samples.

2.4 *Analysis of data*

Data are given as mean ± standard error of the mean (SEM). The significance of mean differences was evaluated using the paired and unpaired two sided *t*-test. Outliers were identified by the Nalimov-test (1972) and removed from the analysis. *P* values less than 0·05 were regarded as biologically significant.

3. Results

Figure 1 shows the $R_{F/G}$ as a function of a single fraction ^{137}Cs-γ-radiation dose of 1, 2, 8 or 16 Gy. The measurements of the $R_{F/G}$, performed at 4, 12 or 24 h after irradiation, have been separately shown. The radiation dose-response curve for the 4 h post-irradiation incubation did not show a significant influence of radiation on $R_{F/G}$. Different doses of radiation affected the $R_{F/G}$ when monitored after 12 and 24 h post-irradiation times. The curves revealed a significant increase of $R_{F/G}$ at 1 Gy (12 h : $P < 0{\cdot}03$), 2 Gy (24 h : $P < 0{\cdot}01$), and 8 Gy (12 h : $P < 0{\cdot}03$; 24 h : P : 0·01).

Figure 2 shows the $R_{F/G}$ as a function of mode of

irradiation. Eight Gy radiation dose was administered either as a single fraction (SF) or as multiple fractions (MF) of 2 Gy each (4 × 2 Gy). The measurements of $R_{F/G}$ were carried out at 12 h post-irradiation time. Whereas $R_{F/G}$ increased significantly ($P < 0.01$) from the control level of 0.99 ± 0.02 (figure $2A_1$) to 1.23 ± 0.05 (figure $2A_2$) after SF, it decreased significantly ($P < 0.02$) to 0.86 ± 0.03 after MF (figure $2A_3$). Figure 2B shows the individual values of the ^{18}FDG utilization and that of the glucose under conditions described in figure 2A.

4. Discussion

In this study the $R_{F/G}$ has been measured in a simplified *in vitro* model. The fractional extraction of the tracer ^{18}FDG and that of 6 mM glucose from the extracellular medium in 45 min represents utilization of these molecules by the monolayer cells in a steady state. Fractions of ^{18}FDG and glucose taken up by the cells might be metabolized in the period of assay spreading over 45 min. Whereas it has been reported for rat and for normal human brain that the entrapped ^{18}FDG and glucose are not likely to be metabolized in this period, nothing is known with certainty for tumour cells (Schmidt *et al* 1996). Since cancerous cells are known to have higher rate of glucose metabolism and the glioblastoma cell line used in this study is a transformed cell line, the assay had to ensure that no erroneous values are measured. By counting ^{18}FDG after washing the cells or by assaying intracellular glucose after 45 min, the loss of ^{18}FDG or glucose due to metabolism could not be accounted for. Therefore, the strategy for measuring the utilization of ^{18}FDG and of glucose was changed in this study. The measure of loss of extracellular ^{18}FDG and glucose from the medium in 45 min, as performed in this study, represents total uptake and metabolism of ^{18}FDG and glucose by cells during this time.

To find out the appropriate condition even for the simplified *in vitro* model, the time of post-irradiation incubation has been verified (figure 1). Measurements done after 12 or 24 h following irradiation resulted in statistically significant increase of $R_{F/G}$ at different doses as compared with the controls. In line with common clinical practice of 12 hourly irradiation of patients in a fractionated dose protocol, 12 h post-irradiation time was selected in this piece of work to measure $R_{F/G}$ after SF and MF modes of irradiation.

The $R_{F/G}$ of unperturbed monolayer cells was found to be 0.99 ± 0.02 (figures 1 and $2A_1$) indicating that the *in vitro* uptakes of ^{18}FDG and glucose were nearly identical. This is quite different from the reported LC values (0·4–0·5) in most brain regions under normal *in vivo* conditions (Gjedde *et al* 1985). The *in vitro* and

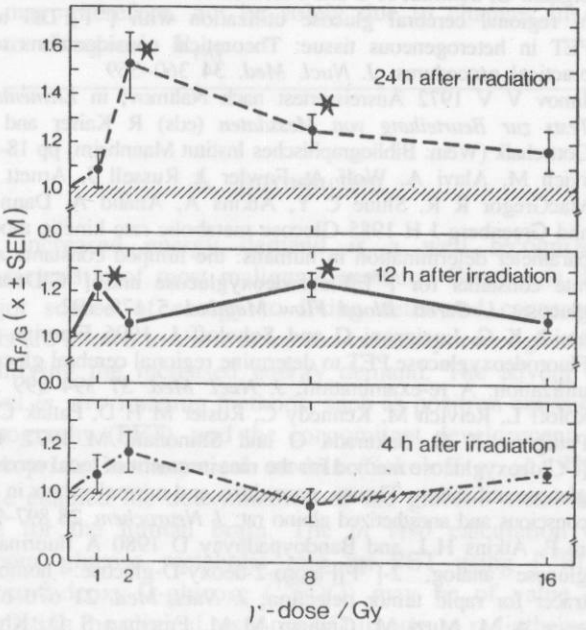

Figure 1. Changes in the $R_{F/G}$ as a function of single fraction ^{137}Cs-γ-irradiation measured at different post-irradiation times. The shaded areas represent the mean $R_{F/G}$ (SEM of 95 non-irradiated monolayer cultures 0.99 ± 0.02) kept under otherwise identical conditions. The radiation induced $R_{F/G}$ which are significantly different to the controls are marked by (*).

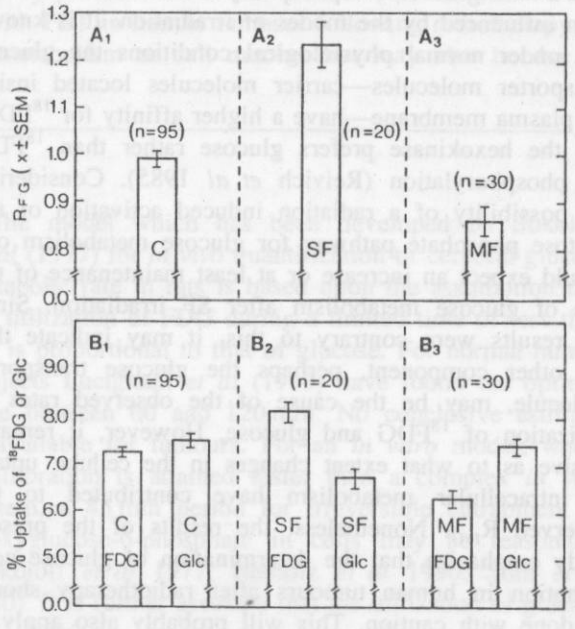

Figure 2. $R_{F/G}$ of non-irradiated control (C) cells (top panel A_1), of cells irradiated to 8 Gy in a single fraction (SF) (top panel A_2) and of cells irradiated to 4 × 2 Gy in a schedule of multiple fractions (MF) (top panel A_3). These values were calculated from the measurements of per cent of ^{18}FDG-over per cent of glucose uptakes by cells from the medium (shown in the bottom panel B_1, B_2 and B_3). All measurements were made at 12 h post-irradiation time.

in vivo situations are different due to organizational difference between 2-dimensional cell monolayer and 3-dimensional tissue, and the physiological difference due to vascularization of tissue which is absent in a cell monolayer. Nonetheless, the $R_{F/G}$ data reported here look similar to the *in vivo* LC values reported by Kapoor *et al* (1989). This seems to support the assumption that $R_{F/G}$ *in vitro* corresponds to the LC *in vivo*.

The *in vitro* study reveals that the $R_{F/G}$ varies with the dose and the mode of γ-irradiation. The radiation induced changes of $R_{F/G}$ (figures 1 and 2) are comparable to the reported LC of four patients with malignant cerebral gliomas, where the LC varied between 0·38 and 0·70 before radiotherapy and between 0·53 and 1·13 after radiotherapy (Spence *et al* 1995). Considering differential uptake of ^{18}FDG and glucose after irradiation (figure 2B), it is interesting to note that the increase of the $R_{F/G}$ after a SF irradiation is due to an increase of ^{18}FDG uptake and a decrease of glucose uptake. After MF irradiation, the decrease of $R_{F/G}$ (figure $2A_3$) was primarily due to the lower ^{18}FDG uptake (figure $2B_3$). This demonstrates that FDG uptake may vary independently of the glucose metabolism indicating that a change in the uptake of FDG after exposure to radiation may not reflect true status of glucose metabolism.

The analysis of the results (figure 2B) suggests that the mode of irradiation influenced the kinetics of ^{18}FDG as well as glucose uptake. The different affinities of ^{18}FDG and glucose for phosphorylation could also have been influenced by the modes of irradiation. It is known that under normal physiological conditions the glucose transporter molecules—carrier molecules located inside the plasma membrane—have a higher affinity for ^{18}FDG, and the hexokinase prefers glucose rather than ^{18}FDG for phosphorylation (Reivich *et al* 1985). Considering the possibility of a radiation induced activation of the pentose phosphate pathway for glucose metabolism one should expect an increase or at least maintenance of the rate of glucose metabolism after SF irradiation. Since our results were contrary to this, it may indicate that the other component, perhaps the glucose transporter molecule, may be the cause of the observed rates of utilization of ^{18}FDG and glucose. However, it remains elusive as to what extent changes in the cellular uptake or intracellular metabolism have contributed to the observed $R_{F/G}$. Nonetheless, the results of the present study emphasize that the determination of glucose consumption in human tumours after radiotherapy should be done with caution. This will probably also apply to chemotherapy (Langen *et al* 1989). Even in a full kinetic modelling approach using a measurement of cerebral blood volume and fitting of individual rate constant of tracer, variations of the $R_{F/G}$ may significantly influence the result of the calculated value of glucose consumption for clinical use.

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References

Gjedde A, Wienhard K, Heiss W-D, Kloster G, Diemer N H, Herholz K and Pawlik G 1985 Comparative regional analysis of 2-fluorodeoxyglucose and methylglucose uptake in brain of four stroke patients with special references to the regional estimation of the lumped constant; *J. Cereb. Blood Flow Metabol.* **5** 163–178

Hamacher K, Coenen H H and Stöcklin G 1986 Efficient stereospecific synthesis of no-carrier-added 2-[^{18}F]fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution; *J. Nucl. Med.* **27** 235–238

Herholz K, Wienhard K and Heiss W-D 1990 Validity of PET studies in brain tumours; *Cerebrovasc. Brain Metabol. Rev.* **2** 240–265

Kapoor R, Spence A M, Muzi M, Graham M M, Abbott G L and Krohn K A 1989 Determination of the deoxyglucose and glucose phosphorylation ratio and the lumped constant in rat brain and a transplantable rat glioma; *J. Neurochem.* **53** 37–44

Langen K-J, Roosen N, Kuwert T, Herzog H, Kiwit J C W, Rota Kops E, Muzik O, Bock W J and Feinendegen L E 1989 Early effects of intra-arterial chemotherapy in patients with brain tumours studied with pet: preliminary results; *Nucl. Med. Commun.* **10** 779–790

Lucignani G, Schmidt K C and Moresco R M 1993 Measurement of regional cerebral glucose utilization with [^{18}F]FDG and PET in heterogeneous tissue: Theoretical considerations and practical procedures; *J. Nucl. Med.* **34** 360–369

Nalimov V V 1972 Ausreissertest nach Nalimov; in *Elementare Tests zur Beurteilung von Messdaten* (eds) R Kaiser and G Gottschalk (Wein: Bibliographisches Institut Mannheim) pp 18–21

Reivich M, Alavi A, Wolf A, Fowler J, Russell J, Arnett C, MacGregor R R, Shiue C Y, Atkins A, Anand A, Dann R and Greenberg J H 1985 Glucose metabolic rate kinetic model parameter determination in humans: the lumped constants and rate constants for [^{18}F]Fluorodeoxyglucose and [^{11}C]Deoxyglucose; *J. Cereb. Blood Flow Metabol.* **5** 179–192

Schmidt K C, Lucignani G and Sokoloff L 1996 Fluorine-18-Fluorodeoxyglucose PET to determine regional cerebral glucose utilization: A re-examination; *J. Nucl. Med.* **37** 394–399

Sokoloff L, Reivich M, Kennedy C, Rosier M H D, Patlak C S, Pettigrew K D, Sakurada O and Shinohara M 1977 The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat; *J. Neurochem.* **28** 897–916

Som P, Atkins H L and Bandoypadhyay D 1980 A fluorinated glucose analog, 2-[^{18}F]Fluoro-2-deoxy-D-glucose: nontoxic tracer for rapid tumor detection; *J. Nucl. Med.* **21** 670–675

Spence A M, Muzi M, Graham M M, Freeman S D, Krohn K A, Link J M, Lewellen T K and OíSullivan F 1995 MRGlc and FDG lumped constant in malignant gliomas: Response to radiotherapy; *J. Cereb. Blood Flow Metabol.* **15** S174

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