PET in Cell Cultures: Oncology, Genetics, and Therapy

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The aim of the studies was to investigate the effects of irradiation and/or chemotherapy in tumor cells and in radiosensitive wild-type mutants as a tool to understand the biological base and mechanisms of modulation of 2-[^{18}F]fluoro-2-deoxy-D-glucose accumulation.

8.1 Introduction

2-[^{18}F]Fluoro-2-deoxy-D-glucose ([^{18}F]FDG) has been shown to be a useful radiopharmaceutical for the quantitative determination of regional glucose metabolism, and it localizes in the brain, heart and in tumors. Higher rates of glucose metabolism have been observed in cancer cells for many years, and the significance of this fact for detection of increased cellular metabolism by [^{18}F]FDG in cancer cells has also been recognized. A group of transport proteins enables glucose and glucose derivatives to enter or leave animal cells (see Chap. 3). [^{18}F]FDG is now the most frequently used radiopharmaceutical in positron emission tomography (PET). Administered doses, scan parameters and evaluation protocols have generally been determined empirically and optimized as a result of pragmatic considerations.

The relationship between administered dose and cumulative concentration in the target organ and in the surrounding tissue (background), on one hand, and the resulting image quality (e.g. detectability of small lesions), on the other hand, depends on the mechanism of [^{18}F]FDG accumulation in cells and the clearance rate of the body by the urogenital system.

When appropriately labeled substrate analogues are used, it is not just the flow of blood or plasma that determines the amount of radioactivity that reaches the tissue but also (and more significantly) the cellular transport and/or intracellular metabolic reactions (e.g. transport and phosphorylation in the case of FDG).

A similarity exists in the metabolic pathways of glucose and of glucose and FDG when the sugars are accumulated by the cell. One phosphate molecule with the alcohol group is added to the sixth carbon atom of a glucose or FDG molecule. This requires the activity of the enzyme hexokinase, which is localized in the cells. In this case glucose and FDG compete for the enzyme binding site. The phosphorylated molecules, glucose-6-phosphate and 2-FDG-6-phosphate, cannot leave the cell. This biochemical pathway of 2-FDG has been called the trapping mechanism (Horton et al. 1973; Gallagher 1978). Due to the trapping mechanism and the increased accumulation in tumors, FDG-6-phosphate can be called a tumor marker.

In patients with cancer, the [^{18}F]FDG method is used for diagnostics and follow-up studies.

In both cases it is important to know whether increased accumulation of ^{18}F activity is caused only by cell proliferation or whether other mechanisms could induce increased uptake as well.

We investigated the dose-dependent change of [^{18}F]FDG uptake in the presence of an irradiation-induced inhibition of cell growth after irradiation under different experimental conditions in different cell culture systems.

8.2 Material and Methods

HeLa cell lines, and wild-type AA8 (not radiation sensitive) or mutant EM9 (radiation sensitive) Chinese hamster cells originated from ATCC, Manassas, Virginia (USA), and [^{18}F]FDG in physiological NaCl solution came from the Forschungszentrum Karlsruhe, or from MPI fuer neurologische Forschung Koeln, Germany).

Cells were irradiated by an X-ray tube (Müller RT 200) with a 200 kV, 0.5 mm Cu filter and a focus distance of 25 cm. These parameters result in a dose rate of 3.75 Gy/min.

Irradiation experiments over a period of several days were started at the onset of the growth phase with 0–30 Gy, with and without chemotherapy, and stopped after 72 hours. For in vitro tumor cell [^{18}F]FDG uptake assays, the cell lines were cultured in plastic Petri dishes containing Dulbecco's Modified Eagle's Medium at 36°C with 10% fetal calf serum. Assays were made by incubations of the monolayer cultures in triplicate with [^{18}F]FDG in phosphate-buffered saline (PBS) buffer, pH 7.2. The reactions were started or stopped by washing the cells three times with PBS buffer.

[^{18}F]FDG uptake was simultaneously determined in a PET gantry, evaluation was made by regions of inter-

est. Cell-bound ^{18}F activity was counted by measurement of the photon emission in the gantry of a Siemens EXACT ECAT 927/47 camera.

8.3 Results

When HeLa cells are incubated with [^{18}F]FDG for 30 minutes, a constant accumulation of ^{18}F activity is observed (Fig. 1). This kind of assay shows that HeLa cells can transport and accumulate [^{18}F]FDG.

Irradiation of HeLa cells shows an inverse relation of cell numbers to increasing X-ray doses (Fig. 2). Proliferation is reduced at 5 Gy, and the cell number decreases at 10–50 Gy.

Accumulation of ^{18}F activity/cell by irradiated cells after 30 minutes incubation with [^{18}F]FDG increased in the cultures within 48 hours. This increase was correlated to the increase of irradiation dose. This can be demonstrated by plotting the relation of ^{18}F accumulation by irradiated cells in relation to the accumulation by the non-irradiated cells (Fig. 3).

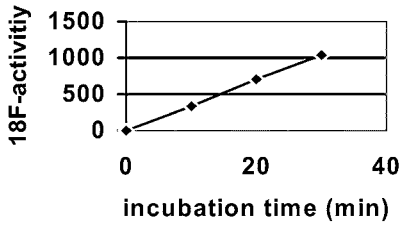

Fig. 1. Accumulation of [^{18}F]FDG in HeLa tissue culture (becquerels/cubic centimeter)

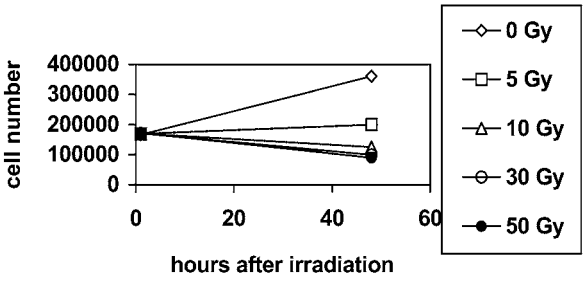

Fig. 2. Effect of irradiation on HeLa cell proliferation

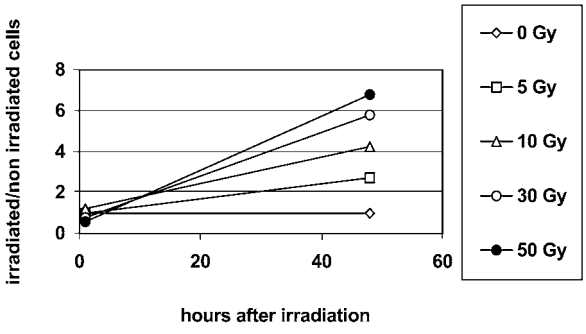

Fig. 3. Effect of irradiation on [^{18}F]FDG accumulation/cell: relationship of irradiated/not irradiated cells (HeLa)

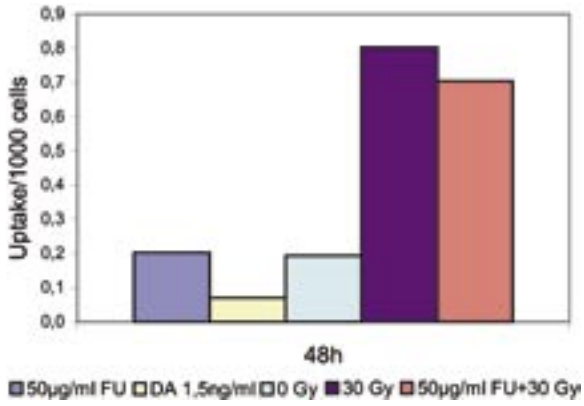

Fig. 4. [^{18}F]FDG uptake (nCi/cc) in EM9 cells (radiation-sensitive mutant cells)

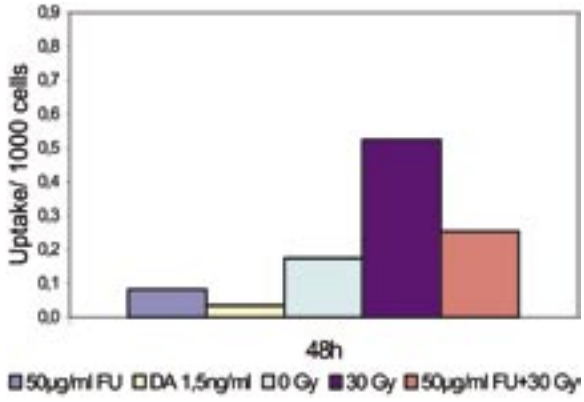

Fig. 5. [^{18}F]FDG uptake (nCi/cc) in AA8 cells (insensitive cells)

Basic data in untreated AA8 and EM9 cells were similar. Irradiation of the cells led to a dose-dependent strong inhibition of cell proliferation, increased ^{18}F accumulation/cell and hexokinase activity/cell, which were higher in radiation-sensitive cells compared to the wild-type (Figs. 4, 5). Chemotherapy could inhibit the irradiation effects.

8.4 Discussion

The clinical significance of [^{18}F]FDG is based on the fact that tumor cells take up more glucose in comparison with normal cells. An increased uptake of [^{18}F]FDG in tumor cells and the detection of this uptake using PET permit a quantitative analysis. The mechanisms of FDG uptake have not yet been fully explained. Discussion centers on whether they are controlled by the prolifer-

ative activity of cells or by other parameters. We investigated the change in [^{18}F]FDG uptake after irradiation with doses of different strengths.

The experimental studies on [^{18}F]FDG uptake in HeLa cells were carried out with FDG concentrations of less than 1 µmol/l and focused on a time period of 30 minutes. Under these conditions, no inhibition of cell growth can be expected. The linearity of the [^{18}F]FDG uptake within a time period of 30 minutes demonstrates that the measurement reflects the period of maximum uptake, and the measured uptake activity reflects the [^{18}F]FDG transport into the cells. In tumor cells, therefore, an increase in the radiation dose brings about an increase in [^{18}F]FDG transport in particular. The dose-dependent change in [^{18}F]FDG uptake is an indication of the proliferation-independent mechanism of [^{18}F]FDG uptake, especially when [^{18}F]FDG uptake increases concomitantly with inhibition of cell division caused by radiation. Since an inflammatory reaction can be excluded as the source of the increased [^{18}F]FDG uptake, it seems obvious to consider repair processes with their increased energy requirements as an explanation for the changed behavior of the cells. The dose-dependent increase suggests an increasing need for repair.

Similar results were obtained with the [^{18}F]FDG PET examination of a patient with two lymph node tumors of a papillary thyroid cancer; the patient received a total dose of 30/60 Gy, respectively. On the basis of this therapy, there was a short-term increase in ^{18}F uptake. Additional examinations featuring an antigranulocytic scan showed an additional increase in [^{18}F]FDG uptake, caused by inflammation, in the area of the metastases (Hautzel et al. 1997).

On the other hand, experimental studies on spheroids from a human adenocarcinoma cell line showed a balance of increased [^{18}F]FDG uptake in surviving cells and an absence of uptake in dead cells after a single irradiation of 6 Gy, with the result that there was no significant change in [^{18}F]FDG uptake per volume unit of the spheroids (Senekowitsch-Schmidtke et al. 1998). In studies involving rats with tumor transplants, it has been observed that after a single irradiation of 10 Gy, radiation-sensitive tumor cells demonstrated a significant two- to threefold increase in the accumulation of ^{18}F within a period of 2–6 hours after irradiation, whereas radiation-insensitive tumors showed an insignificant and low increase of ^{18}F activity (Furuta et al. 1997). If [^{18}F]FDG PET studies are carried out at shorter intervals after the beginning of radiation therapy, clear increases in ^{18}F uptake can be detected. In four patients with intracranial tumors who received 15–27.5 Gy, there was an increase in glucose uptake of 25–42 % 1 day after irradiation; this receded 7 days later and then remained within the range of 10 % above to 12 % below the pretherapeutic basic uptake level (Rozental et al. 1991).

In other studies with 44 and 21 patients, respectively, it was reported that in many patients a decrease in ^{18}F uptake was detectable 3 months after radiation therapy, but that in some cases it was not possible to differentiate between proliferation, repair processes, inflammation and remaining vital tumor tissue (Haberkorn et al. 1991; Engenhart et al. 1992).

8.5 Conclusion

It is possible to detect the dose-dependent increase in [^{18}F]FDG accumulation in HeLa tumor cells using a simple system of cell culture, X-ray tube and PET. It is also possible to investigate these effects in mutants having different radiation sensitivity with and without simultaneous addition of drugs used in therapy of tumors.

This observed increased uptake is triggered by a mechanism independent of cell proliferation, and can presumably be traced to the increased energy requirements of cells during repair processes. These can be differently influenced by drugs interfering with nucleotide or protein synthesis.

Basic data in untreated AA8 and EM9 cells demonstrated that irradiation of the cells led to a dose and genetic disposition-dependent inhibition of cell proliferation. Increased [^{18}F]FDG accumulation/cell and hexokinase activity/cell were higher in radiation-sensitive cells compared to the wild-type. Chemotherapy could inhibit the irradiation effects. As a conclusion, increased irradiation-induced [^{18}F]FDG uptake in the investigated cells may be related to a proliferation-independent increase of hexokinase activity. This uptake increases to levels above those of non-irradiated proliferating tumor cells, is related to the genetic disposition of radiosensitivity and seems to be based on a network of dose- and time-dependent intracellular stress mechanisms, which can be inhibited by chemical drugs. The results give rise to the assumption that there are limits to make reliable FDG PET diagnostics based on the hypothesis of cell proliferation-dependent FDG uptake during radio- and/or chemotherapy.

The user of PET should also be conscious of the fact that the signal he receives from a treated tissue is dependent on the cell number and uptake of radiotracer per cell. PET alone can only refer to the signal of radioactive decay; it provides, however, no information about the cell mass or number of cells. PET-CT may be an approach that partially solves this problem.

From the experimental and clinical studies discussed here, it emerges that three factors can lead to an increase in [^{18}F]FDG uptake after irradiation of tumors in patients: proliferation, inflammation and repair processes. Accordingly, during the [^{18}F]FDG PET examination it must be clarified whether an increased ^{18}F uptake after radiation therapy occurs as a result of the dose, or

whether the tumor tissue has proliferated independent of therapy and more [^{18}F]FDG is being taken up for this reason. It is therefore important to determine the point in time at which an increase in [^{18}F]FDG uptake that is independent of tumor growth no longer appears in patients. Further systematic studies of irradiated cell cultures can provide information on this point.

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