PHYSICS AND RADIATION BIOLOGY

Transient Toxicity of 2-Deoxy-2-[^{18}F]Fluoro-D-Glucose in Mammalian Cells: Concise Communication

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The kinetics of uptake and toxicity of the positron emitter F-18 have been examined in a cultured cell line. 2-Deoxy-2[^{18}F]fluoro-D-glucose (^{18}FDG) concentrated rapidly within Chinese hamster V79 cells, and the uptake was linear with the extracellular radioactive concentrations. Whereas ^{18}FDG synthesized 2 hr before the incubation did not appear to be toxic, that synthesized 5 hr previously was highly toxic. Toxicity was transient and independent of both the extracellular/intracellular radioactive concentration and the energy released from the decay of fluorine-18. Similarly synthesized nonradioactive FDG and Na^{18}F were not toxic under comparable experimental conditions. We conclude that this transient toxicity is due to an unidentified chemical species that is cytocidal following intracellular localization. These toxic levels are not likely to be achieved in the clinical use of ^{18}FDG due to dilution factors that are orders of magnitude greater than those used in these in vitro studies.

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The introduction of fluorine-18 and other positron emitters as intracellular diagnostic agents in nuclear medicine has raised a number of questions regarding the microdosimetry of these radionuclides. Fluorine-18 ($T_{1/2}$ = 110 min) decays to stable oxygen-18, almost entirely (96.9%) by the emission of a positron. As this charged particle traverses matter, it loses its energy very much like a negatron; it slows down and finally interacts with a negatron. The positron-negatron pair will either annihilate in the free state or will form short-lived (<1 μsec) positronium, which will then annihilate and emit two 511-keV photons.

The efficiency of positron emitters in causing cytocidal effects in mammalian systems has not been reported. We have examined the kinetics of uptake and toxicity of 2-deoxy-2-[^{18}F]fluoro-D-glucose (^{18}FDG) in a mammalian cell line in vitro. Our preliminary experiments indicated that 5 hr after synthesis, the toxicity exhibited by ^{18}FDG was far greater than that predicted by classical dosimetric calculations. Surprisingly, this radiopharmaceutical was found to be relatively nontoxic when similar experiments were carried out 2 hr after synthesis. In this paper we describe these experiments as well as other results that indicate the presence of one or more transient toxic species of an unknown nature. Our results further illustrate that this short-lived intermediate (or intermediates) seems to be related to the decay of fluorine-18 and is cytotoxic only when the intermediate (or intermediates) is located within the cell.

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MATERIALS AND METHODS

Cells and media. Chinese hamster V79 lung fibroblasts were maintained in logarithmic growth in plastic 75 cm^2 tissue-culture flasks at 37°C in an atmosphere of 5% CO_2 in air. The cells were grown as monolayers (*1*) in Eagle's minimum essential medium (MEM) supplemented with 15% fetal bovine serum (FBS), 2 m*M* L-glutamine, 100 μ*M* nonessential amino acids, penicillin

(5 units/ml), streptomycin (5 μg/ml), and gentamicin sulfate (50 μg/ml).

F-18-labeled tracers. Fluorine-18-FDG was synthesized by the reaction of 3,4,6-tri-O-acetyl-D-glucal with a F-18-labeled elemental fluorine as described by Fowler et al. (*2*). This procedure typically produced about 1 mg of ^{18}FDG with a specific activity of 20–40 mCi/mg at the end of the synthesis and a radiochemical purity of 96–98%. Similar syntheses were carried out with stable fluorine to produce FDG for use as nonradioactive controls.

Fluorine-18-labeled sodium fluoride was prepared by the $^{16}O(^3He,p)^{18}F$ reaction using a water target and adding dry sodium chloride to give an isotonic solution.

Uptake studies. To determine the uptakes of ^{18}FDG and Na^{18}F, V79 cells grown in monolayers were trypsinized, suspended in calcium-free MEM (4×10^5 cells/ml), dispensed into sterile plastic test tubes, and incubated at 37°C for 4 hr (*3*). Various radioactive concentrations of ^{18}FDG or Na^{18}F were added to each tube (8×10^5 cells/4.0 ml) and the cells reincubated at 37°C for 220 min on an orbital shaker. Despite continuous agitation of the tubes, the cells sedimented to a volume ~0.2 ml within ~30 min. Cellular uptake of radioactivity was measured by the microfuge separation technique (*3,4*). Briefly, the cells were sedimented by centrifugation at 2000 rpm for 5 min and 100-μl aliquots from the supernatants were layered onto the surface of 300 μl of FBS in 400-μl microfuge tubes. Ten microliters of each supernatant were also blotted onto filter paper to assess the extracellular radioactive concentration present in the medium. The pelleted cells in the test tubes were then resuspended and 100 μl of the cell suspensions were transferred into microfuge tubes containing FBS. The microfuge tubes were spun at 12,800 *g* for 1 min in an Eppendorf microfuge, frozen, and the radioactive contents present at the tube tips measured in an autogamma counter. All counts were corrected for decay back to the time the radioactivity was added to the cells. Counting efficiency, as determined by spotting known volumes of standard solutions of F-18 onto filters, was found to be 34%.

Survival assay. The toxicities of FDG, NaF, Na^{18}F, ^{18}FDG, and their decayed product(s) were determined by the colony-forming assay (*1*). In this assay, an adequate number of cells were seeded into plastic 25 cm^2 T-flasks, in triplicate, to yield 30–250 colonies at seven days after exposure to each compound. Control flasks for each experiment contained unexposed cells treated similarly. The colonies formed were fixed in Bouin's fixative, stained with trypan blue and air-dried. The ability of each cell to form a single colony of 50 or more cells was considered to indicate survival. Colonies were counted, and the mean and s.e.m. of each triplicate were determined. Survival fractions (S/So) were calculated relative to control cultures (plating efficiency of exposed cells divided by plating efficiency of unexposed cells). The toxicity of each compound was then determined by plotting S/So as a function of the mean radioactivity associated with the cells.

Dosimetric calculations. The following was obtained from MIRD tables for fluorine-18 (*5*):

Half-life = 1.83 hr, positron range (90%) = 470 μm.

Radiation	N_i	E_i (*MeV*)	Δ_i (*g-rad/μCi-hr*)
β^+	0.97	0.2496	0.5157
Ann. rad. (AR)	1.94	0.5110	2.1115

Absorbed fraction of annihilation photons (ϕ_{AR}) for 0.2-ml ellipsoids = 0.009.

For the geometry used in these experiments (800,000 cells, 5.14 μm in radius, dispersed in ~0.2 ml of ^{18}FDG-containing medium), and with the intracellular concentration of ^{18}FDG two orders of magnitude greater than the extracellular, the dosimetry has two components: the irradiation of cells by their own intracellular F-18, and the irradiation of cells by F-18 from the medium and from other cells. Because of the distance between cells and the small total amount of F-18 in the cells relative to the medium, the irradiation of one cell by others can be neglected or approximated by a uniform distribution of radioactivity outside of each cell.

Thus the general dose term can be written

$$D = \tilde{A}_e(\Delta_{\beta+} + \phi_{AR}\Delta_{AR}) + \tilde{A}_i(\phi_{\beta+}\Delta_{\beta+}),$$

where the first term on the right expresses the radiation dose to cells from the external medium (plus other cells), and the second term is the radiation dose to cells from themselves.

$\tilde{A}_e$ = cumulative activity per ml in medium,

$\tilde{A}_i$ = cumulative activity per ml in cells.

These are calculated as:

$$\tilde{A}_j = \frac{C_{oj}}{\lambda}(1 - e^{-\lambda t}),$$

where C_{oj} is the initial concentration and $\tilde{A}_j$ is the cumulative activity in medium or cells.

For t = 220 min: $\tilde{A}_j = 1.98\ C_{oj}$.

The value of $(\Delta_{\beta+} + \phi_{AR}\Delta_{AR}) = 0.5157 + (0.009)(2.115) = 0.535$, and of $(\phi_{\beta+}\Delta_{\beta+}) \cong (5.14)(0.5157)/470 = 0.0056$.

Hence the dose delivered to the cells is given by

$$D_{220} = 1.059\ C_{oe} + 0.011\ C_{oi}.$$

RESULTS

Kinetics of ^{18}FDG and Na^{18}F uptake. The rate of up-

take of ^{18}FDG by V79 cells following varying incubation periods at 37°C is shown in Fig. 1. Uptake (pCi/cell) is plotted as a function of the duration of incubation (hr). Fluorine-18-FDG uptake, as measured by the microfuge method (*4*), was found to be most rapid in the first hour and reached a plateau by 3 hr. These results were confirmed when V79 cells were incubated with various radioactive concentrations of ^{18}FDG for 220 min and uptake was plotted as a function of extracellular radioactivity (Fig. 2). Uptake was linear with increasing radioactive concentrations (slope = 0.026) and exhibited a correlation coefficient of more than 0.98. As expected, the incubation of V79 cells with Na^{18}F did not result in any measurable uptake.

Clonal survival and dosimetry. The survival curves of V79 cells following a 220-min incubation with ^{18}FDG 5 hr after synthesis are illustrated in Fig. 3A. Over the ranges observed, the curve was characterized by an initial broad shoulder and a logarithmic portion. Using the mean extracellular radioactive concentration at 37% survival as a relative index of toxicity, the lethal dose of ^{18}FDG was 58 μCi/ml, with a corresponding intracellular radioactive content of 1.7 pCi/cell.

When these external and internal concentrations were used to calculate the dose received by the cells, a value of 94 rads was obtained. This is considerably less than the x-ray D_{37} for these cells, which is 580 rads (*1*), and it suggested to us that factors other than radiation were responsible for the toxicity observed.

Further experimentation using ^{18}FDG 2 hr after synthesis demonstrated that while the rates of uptake by V79 cells were similar to those reported for 5-hr postsynthetic ^{18}FDG (Fig. 2), the toxicity of the compound was considerably less (Fig. 3A). This was a further indication that the toxicity observed earlier was not directly related to the radioactive concentration of ^{18}FDG.

The discrepancy between the 2-hr and the 5-hr postsynthetic toxicity experiments was resolved when it was noted that as a result of the short half-life of F-18 (110 min), the volumes of the stock ^{18}FDG solutions used in the 5-hr runs were about 3.1 times those used in the 2-hr experiments. As a result, a plot of the survival data as a function of the volume of the stock ^{18}FDG solution produces a single survival curve (Fig. 3B).

In addition, plotting the survival of V79 cells in the presence of a fixed volume of ^{18}FDG stock solution as a function of varying postsynthetic time, indicates that the toxicity of the ^{18}FDG solution decreases with time in a linear logarithmic manner. It appears that the toxicity decreases rapidly from the fifth hour on.

Finally, the incubation of V79 cells with decayed ^{18}FDG (>24 hr after synthesis), or with cold FDG at equimolar concentrations, did not cause any measurable toxicity, nor did the incubation with equivalent radioactive concentrations of Na^{18}F.

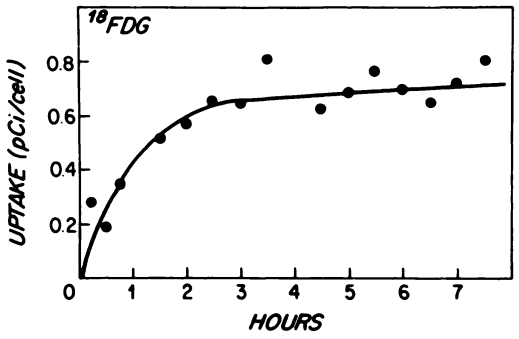

FIG. 1. Uptake of ^{18}FDG by Chinese hamster V79 lung fibroblasts as function of incubation period. Cells were incubated with ^{18}FDG (30 μCi/ml) and cell-associated activity assayed after various periods by microfuge method (*4*).

DISCUSSION

The experiments demonstrate the ability of Chinese hamster V79 cells to accumulate ^{18}FDG in concentrations 50 times that of the surrounding medium. The uptake is moderately rapid, appears to obey Michaelis-Menten kinetics, and reaches a plateau at about 2 hr. These observations are in keeping with those made in other biological systems (*6,7*).

In the suspensions used, where the cells occupy less than 0.5% of the volume (the distance between cells, ~50 μm, being greater than the cell diameters, 10 μm, yet considerably smaller than the range of the positrons), the intracellular fluorine-18 component contributes about 1/3 to the radiation dose received by individual cells. In packed cells and tissues, where the cells occupy ~70% of the volume, the major component is the irradiation of one cell by others surrounding it, out to about 5 cell diameters. Under these circumstances, the MIRD approximation of a homogeneous distribution of radioac-

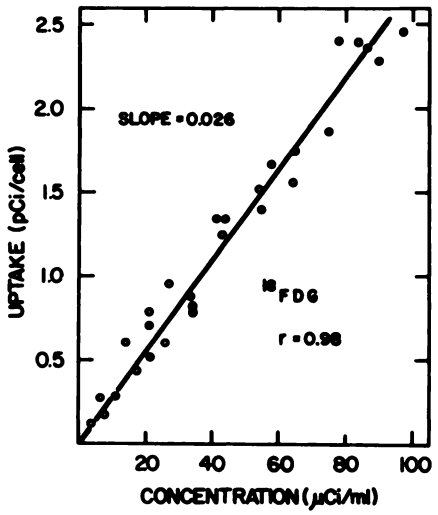

FIG. 2. Uptake (pCi/cell) of ^{18}FDG by V79 cells as function of extracellular radioactive concentration (μCi/ml). Incubation period 220 min; postsynthesis times: 2 hr (●), 5 hr (○).

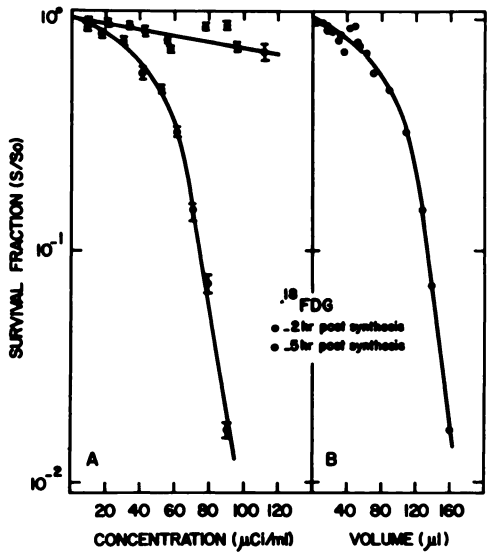

FIG. 3. A. Survival of V79 cells incubated for 220 min with varying radioactive concentrations of ^{18}FDG, starting 2 hr (●) and 5 hr (O) after synthesis. The survival fraction (S/So), determined by the colony forming assay (*1*), is plotted as function of extracellular concentration (μCi/ml). Each point represents mean of three replicate flasks ±s.e.m. B. Survival data from Fig. 3A plotted as function of volume of stock ^{18}FDG solution used.

tivity, assuming a weighted average concentration between cells and extracellular fluid, applies with very little error.

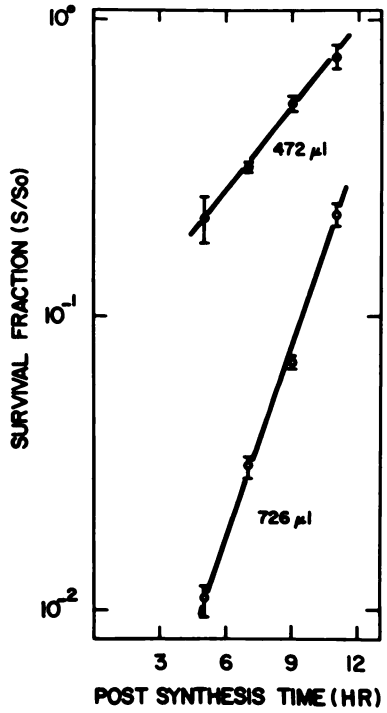

FIG. 4. Survival of V79 cells incubated in two constant volumes of ^{18}FDG solution for 220 min, as function of postsynthesis time (hr). Each point represents average of three replicate flasks ±s.e.m. Volumes used: 472 μl [●] and 726 μl [O], with corresponding radioactive concentrations of 108 and 172 μCi/ml at 5 hr after synthesis.

The varying toxicities observed following the incubation of V79 cells with ^{18}FDG 2 to 14 hr after the compound has been synthesized (Figs. 3 and 4) indicate that these cytocidal effects are not due to the energy released from the decay of fluorine-18. The transient nature of the toxicity suggests that it is due to either an unstable chemical product formed from the reactants in the synthesis of ^{18}FDG or a radionuclide that "grows in" and then decays. However, pulse-height analysis of the cyclotron products showed no evidence for contaminating radioactive species. We conclude, therefore, that the transient toxicity is due to some unknown chemical species that is cytocidal only following intracellular localization. This conclusion is based on the observations that the toxic species is not a product of the synthetic process forming ^{18}FDG, since similarly synthesized nonradioactive FDG is not toxic, and that Na^{18}F, which remains extracellular, and its decay product(s), are not toxic.

The nature of the toxic species has not yet been determined. The facts that it is formed following synthesis and is absent in 24-hr-old material is evidence for its instability. The substance may be formed from other bio-organic substances through radiolysis initiated by the positron, or more likely, the positron decay of the fluorine-18 atom leads to a highly unstable transient molecule. The decay of fluorine-18 leads to stable oxygen-18, but the modest recoil imparted to the oxygen as it is formed probably leads to bond rupture with a charged, unstable deoxy-sugar left behind. This molecule can fragment or rearrange due to the excitation energy and charge it holds (*8*). Thus the cytotoxic substance may be related to this unstable intermediate or the fragmentation products that may be formed subsequently. Further studies are needed to clarify these possibilities.

Finally, since the dilution factors achieved in the clinical use of ^{18}FDG are orders of magnitude greater than those used in the in vitro system described here, it is highly unlikely that the clinical use of ^{18}FDG will have any adverse effects in human studies. This is supported by the fact that no adverse effects have been observed since the inception of the ^{18}FDG method in 1976, with thousands of studies since carried out worldwide.

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