[Chem. Pharm. Bull. 36(3)1194—1197(1988)]

Metabolic Pathway of 2-Deoxy-2-fluoro-D-glucose and 2-Deoxy-2-fluoro-D-mannose in Mice Bearing Sarcoma 180 Studied by Fluorin-19 Nuclear Magnetic Resonance

MASAHARU KOJIMA,*,[a] SATORU KURIBAYASHI,[a] YOKO KANAZAWA,[a] TERUSHI HARADAHIRA,[a] YOSHIHIKO MAEHARA,[b] and HIDEYA ENDO[c]

Faculty of Pharmaceutical Sciences,[a] Cancer Center of University Hospital,[b] and Medical Institute of Bioregulation,[c] Kyushu University, Maidashi 3–1–1, Higashi-ku, Fukuoka 812, Japan

(Received July 18, 1987)

The metabolic products of 2-deoxy-2-fluoro-D-glucose (FDG) and 2-deoxy-2-fluoro-D-mannose (FDM) in sarcoma 180 cells transplanted in mice were investigated by fluorin-19 nuclear magnetic resonance (^{19}F-NMR) spectroscopy. It became apparent that the administered FDG was converted to FDM (and/or FDM-6-phosphate) in tumor cells, and also the administered FDM was converted to FDG (and/or FDG-6-phosphate). At 9 h after administration of FDM, the ratio of FDG (and/or FDG-6-phosphate) and FDM (and/or FDM-6-phosphate) reached equilibrium. On the other hand, it took more than 48 h in the case of FDG administration. The equilibrium amount of FDM (and/or FDM-6-phosphate) was approximately four times as much as that of FDG (and/or FDG-6-phosphate) in both cases.

Keywords——2-deoxy-2-fluoro-D-glucose; 2-deoxy-2-fluoro-D-mannose; ^{19}F-NMR; sarcoma 180; tumor; metabolism; epimerization; *in vivo* NMR

2-Deoxy-D-glucose (DG) or 2-deoxy-2-fluoro-D-glucose (FDG) labeled with suitable radionuclide is well known as a tracer for glucose uptake and phosphorylation in cells of organs or tissues.[1] Each compound is incorporated into cells in the same manner as glucose and is converted to DG-6-phosphate (DG-6-P) or FDG-6-phosphate (FDG-6-P) catalyzed by hexokinase, which is active for not only glucose but also DG and FDG.[2] However, it has been considered that DG-6-P and FDG-6-P are not further metabolized in the glycolytic pathway because they are not substrates for phosphohexose isomerase, and, as a result, the administered DG or FDG accumulates in cells as DG-6-P or FDG-6-P.[3] In fact, this phenomenon has been applied for the calculation on local organ glucose metabolism, and also for imaging of organs of tissues such as brain or tumor by positron emission tomography (PET) using a radiotracer such as ^{18}F-FDG.[4] Recently, research on the metabolism of FDG and 2-deoxy-2-fluoro-D-mannose (FDM) by fluorin-19 nuclear magnetic resonance (^{19}F-NMR) spectroscopy has begun. Namely, Nakada *et al.*[5] suggested that FDG administered to rats is metabolized to 2-deoxy-2-fluoro-6-phospho-glucono-δ-lactone and/or 2-deoxy-2-fluoro-6-phospho-gluconate through FDG-6-P in the pentose monophosphate shunt in the brain. On the other hand, Kanazawa *et al.* showed that the FDG administered to mice is metabolized to FDM (and/or FDM-6-phosphate (FDM-6-P)) in the brain and heart,[6] and also administered FDM is metabolized to FDG (and/or FDG-6-P).[7]

It is known that cancer cells consume much larger quantities of glucose as an energy source than normal cells.[8] Accordingly, we tried to examine the metabolic products of FDG and FDM in sarcoma 180 cells in mice by ^{19}F-NMR spectroscopy.

Experimental

Materials——FDG and FDM were prepared by the methods reported earlier.[9,10] FDG-6-P was prepared according to the method of Bessell and Thomas.[3] The values of coupling constants of FDG-6-P in the ^{19}F-NMR spectrum were consistent with those reported. Essentially the same method was used to obtain FDM-6-P as the sodium salt. ^{19}F-NMR (D_2O, external reference C_6F_6, 93.7 MHz) δ: +37.3 ppm ($J_{1,F}=8$ Hz, $J_{2,F}=52$ Hz, $J_{3,F}=31$ Hz, α-anomer), δ: +55.7 ppm ($J_{1,F}=20$ Hz, $J_{2,F}=52$ Hz, $J_{3,F}=32$ Hz, β-anomer).

Animal Experiments——Five-week-old ddY mice (25—30 g weight) were inoculated with about 1×10^7 sarcoma 180 cells intraperitoneally. After a week, sarcoma 180-bearing mice were fasted for 16 h. A fluorohexose (0.2 g/kg) dissolved in isotonic saline was injected into the mice through the tail vein, and abdominal ascites was withdrawn at various time intervals. The abdominal ascites fluid was centrifuged at $900\times g$ for 5 min at 4 °C, and the precipitated cells were washed with isotonic saline twice to remove contaminating blood by centrifugation at $400\times g$ for 5 min at 4 °C. The pellets thus obtained were boiled for 3 min and frozen until ^{19}F-NMR measurements (Fig. 1).

^{19}F-NMR Measurements——^{19}F-NMR spectra of sarcoma 180 cells were measured at 93.7 MHz on a JEOL FX-100 spectrometer in a 10 mm sample tube at 25±1 °C. Hexafluorobenzene (100%) was used as an external standard for chemical shift measurements. T_1 of FDG (and/or FDG-6-P) and of FDM (and/or FDM-6-P) signals in boiled sarcoma 180 cells had the same value of 1.1±0.1 s at 25°C. NMR measurements and the determination of fluorohexoses concentrations in cells were done according to the method in the literature.[6]

Results

The ^{19}F-NMR spectrum of treated sarcoma 180 cells showed three signals corresponding to the chemical shifts of α,β-FDG (and/or α,β-FDG-6-P), α-FDM (and/or α-FDM-6-P) and β-FDM (and/or β-FDM-6-P) (Table 1). The differences of chemical shifts of signals shown by the fluorohexoses and the respective 6-phosphates in tissues were too small to be distinguished.

Figure 2a shows the relative amounts of FDG (and/or FDG-6-P) and FDM (and/or

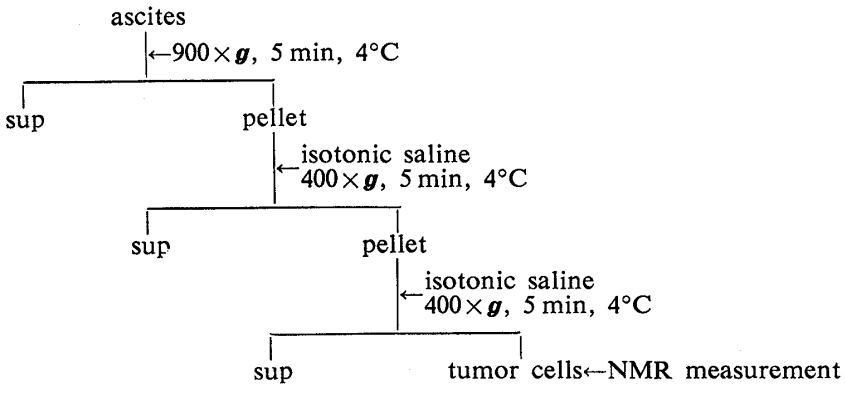

Fig. 1. Separation of Tumor Cells

TABLE I. ^{19}F Chemical Shifts

Sample	Anomeric form	Chemical shift (ppm)[a]	Sample	Anomeric form	Chemical shift (ppm)[a]
FDG	α	−32.3	FDM-6-P	α	−37.3
	β	−32.2		β	−55.7
FDG-6-P	α	−32.4	FDG injected sarcoma 180		−32.3 −37.4 −55.7
	β	−32.2			
FDM	α	−37.6	FDM injected sarcoma 180		−32.2 −37.4 −55.9
	β	−56.0			

a) Referred to an external standard of hexafluorobenzene (HFB).

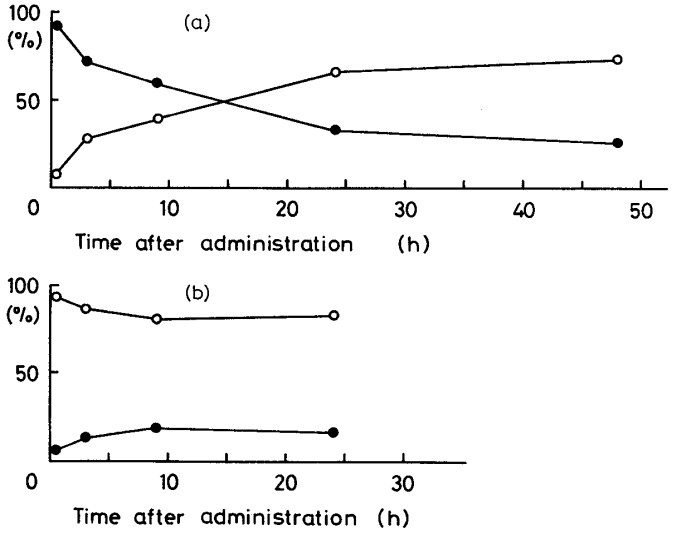

Fig. 2. Relative Amounts of FDG (and/or FDG-6-P) and FDM (and/or FDM-6-P) in Sarcoma 180

●, FDG (and/or FDG-6-P); ○, FDM (and/or FDM-6-P).

(a) FDG administration: Each point represents the average of 9 mice. (b) FDM administration: Each point represents the average of 4 mice. The ordinate shows the ratio (%) of FDG (and/or FDG-6-P) and FDM (and/or FDM-6-P) to total fluorohexoses measured by ^{19}F-NMR in sarcoma 180 cells.

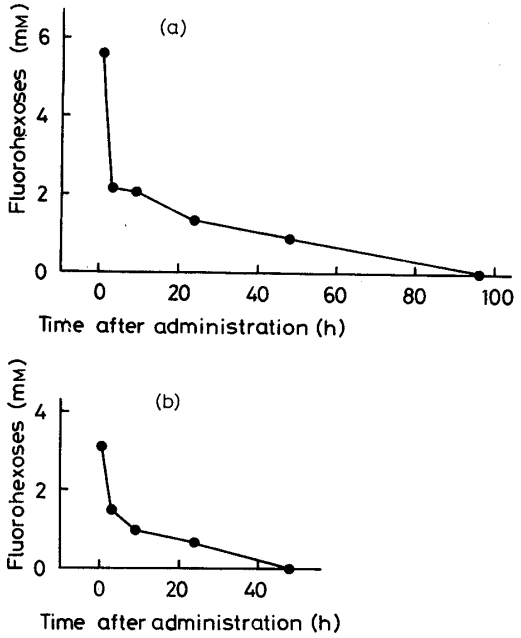

Fig. 3. Uptake of Fluorohexoses in Sarcoma 180 Cells Determined by ^{19}F-NMR

(a) FDG administration: Fluorohexoses were detected in sarcoma 180 cells of 4 mice after 48 h but were not detected after 96 h. (b) FDM administration: The fluorohexoses were not detected at 48 h. The concentrations of fluorohexoses were determined in the packed cells collected by centrifugation of $400 \times g$ for 5 min. The limit of detection of the fluorohexoses by ^{19}F-NMR was 0.015 mM. For other conditions, see the legend to Fig. 2.

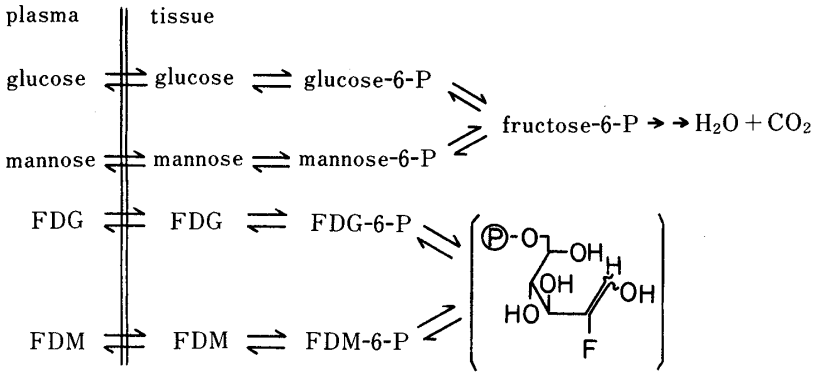

Fig. 4. Proposed Metabolic Pathway

FDM-6-P) in the sarcoma 180 cells withdrawn at 0.5, 3, 9, 24, and 48 h after administration of FDG. Each point represents the mean of 9 mice. It can be seen that the value of FDM (and/or FDM-6-P) increased with time and exceeded that of FDG (and/or FDG-6-P) at 24 h after administration of FDG.

Figure 2b also shows the relative amounts of FDG (and/or FDG-6-P) and FDM (and/or FDM-6-P) in the sarcoma 180 cells withdrawn after administration of FDM. Each point represents the mean of 4 mice. FDG (and/or FDG-6-P) in cells increased with time, and the ratio of FDG (and/or FDG-6-P) to FDM (and/or FDM-6-P) is approximately 1 : 4 at 9 h after administration of FDM. These phenomena are similar to the FDG (and/or FDG-6-P)⇌FDM (and/or FDM-6-P) conversion in the brain or heart, as reported earlier.[6,7]

However, accumulation of fluorohexoses in sarcoma 180 at 30 min after administration of FDG reached 2.6 and 6.0 times those in the brain and heart respectively, while at 30 min after administration of FDM the corresponding values were 2.1 and 8.0 times, respectively. The concentration of all of the fluorohexoses in the sarcoma 180 cells gradually decreased with time, and no signals of the fluorohexoses were detected at 96 h after administration of FDG (Fig. 3a), and at 48 h after administration of FDM (Fig. 3b).

Discussion

Conversion of FDG to FDM or *vice versa* has already been recognized in the brain and other organs of mice, as reported in the previous paper.[6,7] Interconversion between FDG (and/or FDG-6-P) and FDM (and/or FDM-6-P) in sarcoma 180 cells was demonstrated in our present experiments. A hypothetical pathway of the interconversion between FDG and FDM is illustrated in Fig. 4. It is well known that glucose-6-phosphate produced from glucose by hexokinase or glucokinase is converted to fructose-6-phosphate by phosphoglucose isomerase through an intermediate enediol, followed by further metabolism.[11] Our findings may suggest that FDG-6-P formed from FDG by hexokinase is also a substrate for phosphoglucose isomerase, but as the C-2 is replaced with fluorine atom, FDG-6-P is unable to be metabolized in the glycolytic pathway and, as a result, undergoes conversion to FDM-6-P through a 1,2-double bond intermediate. The conversion of FDM to FDG should proceed similarly, in the opposite direction. However, the question why FDM-6-P is produced in a greater amount than FDG-6-P from the 1,2-double bond intermediate still remains to be solved.

Acknowledgment This work was supported in part by a Grant-in-Aid for Scientific Research (No. 61010040) from the Ministry of Education, Science and Culture.

References

1) L. Sokoloff, M. Reivich, C. Kennedy, M. H. Des Rosiers, C. S. Patlak, K. D. Pettigrew, and M. Shinohara, *J. Neurochem.*, **28**, 897 (1977); D. Kuhl, M. Reivich, A. Wolf, J. Greenberg, M. Phelps, T. Ido, V. Casella, J. Fowler, B. Gallagher, E. Hoffman, A. Alavi, and L. Sokoloff, *J. Nucl. Med.*, **18**, 614 (1977); M. Reivich, A. Wolf, J. Greenberg, M. Phelps, T. Ido, V. Casella, J. Fowler, E. Hoffman, A. Alavi, P. Som, and L. Sokoloff, *Circ. Res.*, **44**, 127 (1979); S. C. Huang, M. E. Phelps, E. J. Hoffman, and D. E. Kuhl, *J. Cereb. Blood Flow Metabol.*, **1**, 391 (1981).
2) B. M. Gallager, J. S. Fowler, N. I. Gutterson, R. R. MacGregor, C. N. Wan, and A. P. Wolf, *J. Nucl. Med.*, **19**, 1154 (1978).
3) E. M. Bessell and P. Thomas, *Biochem. J.*, **131**, 77 (1973).
4) B. M. Gallager, A. Ansari, H. Atkins, V. Casella, D. R. Christman, J. S. Fowler, T. Ido, R. R. MacGregor, P. Som, C. N. Wan, A. P. Wolf, D. E. Kuhl, and M. Reivich, *J. Nucl. Med.*, **18**, 990 (1977); M. E. Phelps, E. J. Hoffman, C. Selin, S. C. Huang, G. Robinson, N. MacDonard, H. Schelbert, and D. E. Kuhl, *ibid.*, **19**, 1311 (1978); P. Som, H. L. Atkins, D. Bandoypadhyay, J. S. Fowler, R. R. MacGregor, K. Matui, Z. H. Oster, D. F. Sacker, C. Y. Shiue, H. Turner, C. N. Wan, A. P. Wolf, and S. V. Zabinski, *ibid.*, **21**, 670 (1980).
5) T. Nakada, I. L. Kwee, and C. B. Conboy, *J. Neurochem.*, **46**, 198 (1986).
6) Y. Kanazawa, Y. Momozono, M. Ishikawa, T. Yamada, H. Yamane, T. Haradahira, M. Maeda, and M. Kojima, *Life Sci.*, **39**, 737 (1986).
7) Y. Kanazawa, Y. Momozono, H. Yamane, T. Haradahira, M. Maeda, and M. Kojima, *Chem. Pharm. Bull.*, **35**, 895 (1987).
8) E. Kun, P. Talalay, and H. G. Williams-Ashman, *Cancer Res.*, **11-II**, 855 (1951).
9) T. Haradahira, M. Maeda, Y. Kai, H. Omae, and M. Kojima, *Chem. Pharm. Bull.*, **33**, 165 (1985).
10) T. Haradahira, M. Maeda, H. Omae, Y. Yano, and M. Kojima, *Chem. Pharm. Bull.*, **32**, 4758 (1984).
11) I. A. Rose and E. L. O'Connell, *J. Biol. Chem.*, **236**, 3086 (1961).