Membolic Imaging of Glycoconjugate Synthesis in Tumors with PET Using 6-[18F]Fluoro-L-Fucose

III. 4. Metabolic Imaging of Glycoconjugate Synthesis in Tumors with PET Using 6-[^{18}F]Fluoro-L-Fucose

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Introduction

Radiolabeled L-fucose is used as a selective precursor of the glycoprotein synthesis of the cellular plasma membrane. In previous work, we have found that 6-[^{18}F]fluoro-L-fucose (6-^{18}FFuc) is applicable for tumor imaging with positron emission tomography (PET).[1] In this paper tumor uptake of 6-^{18}FFuc, its metabolism and tumor imaging with autoradiography and PET are investigated using several tumor models.

Materials and Methods

For tumor uptake studies of 6-^{18}FFuc, rats bearing AH109A hepatoma, Yoshida sarcoma (YS), and KEG-1 glioma, and mice bearing FM3A carcinoma and Lewis lung carcinoma (3LL) were used. Tissue uptake of radioactivity was expressed as the differential absorption ratio (DAR), i.e. (count/g tissue)×(g body weight/total injected counts).

Metabolites of 6-^{18}FFuc was analyzed by the method described previously.[2] Briefly, the plasma, liver and tumor samples obtained at 60 min were homogenized in $HClO_4$ solution and divided in the acid-soluble and acid-insoluble fractions. The acid-soluble fraction was analyzed by HPLC.

A mixture of 6-^{18}FFuc and [1-^{14}C]-L-fucose (^{14}C-Fuc) was injected into mice bearing 3LL on the back and in the lung or 3H/He mice bearing spontaneous hepatomas. At 60 min after injection respective, whole body autoradiograms of ^{18}F and ^{14}C were made.[3]

A rabbit bearing VX2 in the right hind leg was injected i.v. with 6-^{18}FFuc, and the distribution of radioactivity was measured tomographycally for 60 min using a positron camera (PT931).

Results

The five types of tumors showed different time-radioactivity patterns (Table 1). The uptake of 6-^{18}FFuc in 3LL tumor was high and maintained a steady level for 2 h. In the FM3A, the radioactivity decreased for the first hour, and maintained constant for the following hour. In three other tumors, the radioactivity decreased with time.

In the FM3A, liver and plasma high percentages of radioactivity were detected in the acid-insoluble fraction; 65% for FM3A, 41% for liver and 74% for plasma. Fig. 1 shows the results of the HPLC analysis of acid-soluble metabolites. In the FM3A, GDP-6-^{18}FFuc was the major metabolite in the acid-soluble fraction. A small amount of 6-^{18}FFuc-1-phosphate was also observed. In the liver a third metabolite, 6-^{18}F-fluorofuconate, was also present. A small amount of a metabolite with a retention time of 16 min was not identified.

Effects of L-fucose loading on the tissue distribution and metabolism were studied in FM3A-bearing mice (Tables 2 and 3). The uptake in all tissues investigated in the L-fucose loading groups was significantly lower than that of the control. Also the ratios of acid-precipitated radioactivity were reduced by the L-fucose loading.

The autoradiograms of 6-^{18}FFuc and ^{14}C-Fuc in a 3LL-bearing mouse and in a mouse with spontaneous hepatomas are illustrated in Figs. 2 and 3, respectively. Clear tumor images using both radiolabeled fucoses were demonstrated. In a 3LL-bearing mouse a higher tumor image using 6-^{18}FFuc by contrast with the surrounding normal tissues was obtained when compared to ^{14}C-Fuc. The 3LL nodules (an experimental metastatic model) in the lung showed a higher density of both tracers than 3LL (a primary tumor model) in the lung showed a higher density of both tracers than 3LL (a primary tumor model) on the back. Table 4 shows the uptake ratios of 6-^{18}FFuc and ^{14}C-Fuc in the tumors and liver. The 3LL metastatic model showed a greater ratio than the 3LL primary model.

The VX2 image with PET is illustrated in Figure 4. A clear image emphasizing the contrast between the central necrosis and the surrounding vital tumor tissue was obtained. The tumor-to-muscle and tumor-to-blood radioactivity ratios were 3.3 and 1.2, respectively, 60 min after injection.

Discussion

The significance for developing a positron-emitting L-fucose analogue is based on the glycoprotein synthesis in plasma membranes. The plasma membrane is involved in a number of tumor cell properties such as cell growth, division, movement, communication, differentiation and antigenicity. Several results demonstrate that 6-^{18}FFuc is a biological active L-fucose analogue. The 6-^{18}FFuc taken by tissues was phosphorylated, activated to GDP form as a sugar donor and incorporated into glycoconjugate macromolecules as a parent L-fucose is.[4] In the liver the oxidation pathway is also observed.[5] The plasma glycoproteins synthesized *de novo* in the liver appeared in the blood.[6] The tracer competed with L-fucose: both the uptake and the ratios of the acid-insoluble radioactivity were significantly reduced by L-fucose loading. In the autoradiographic studies very similar distributions of 6-^{18}FFuc and ^{14}C-Fuc were demonstrated in the same animals. The different time-radioactivity patterns of 6-^{18}FFuc in five types of tumors and a higher accumulation in the metastatic 3LL model than the primary model suggest that the 6-^{18}FFuc has a potential to diagnose the cancer types in various tumors with different glycoconjugate synthesis rates.

In conclusion, 6-^{18}FFuc has a potential as a tracer assessing *in vivo* glycoconjugate synthesis in tumors with PET.

References

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Table 1. Tumor uptake of 6-[^{18}F]fluoro-L-fucose in tumor-bearing rats and mice after i.v. injection.

	Uptake (DAR)*		
	30 min	60 min	120 min
AH109A	0.31±0.04	0.26±0.02	0.21±0.02
YS	0.39±0.07	0.24±0.04	0.23±0.04
KEG-1	0.41±0.05	0.31±0.01	0.25±0.04
3LL	0.45±0.12	0.57±0.19	0.55±0.19
FM3A	0.43±0.10	0.32±0.01	0.35±0.05

*Mean ± s.d. (n=3-7)

Table 2. Effects of L-fucose loading on tissue distribution of 6-[^{18}F]fluoro-L-fucose 60 min after i.v. injection.

	Uptake (DAR)*		
	Control	L-Fucose loading groups**	
		5 mg	50 mg
Blood	0.58±0.16	0.27±0.06 (0.46)	0.21±0.02 (0.36)
FM3A	0.92±0.29	0.34±0.03 (0.37)	0.30±0.05 (0.32)
Brain	0.07±0.01	0.07±0.01 (0.96)	0.07±0.02 (0.96)
Heart	0.35±0.05	0.27±0.03 (0.78)	0.25±0.03 (0.72)
Lung	0.48±0.06	0.30±0.03 (0.62)	0.25±0.03 (0.51)
Liver	0.81±0.15	0.28±0.03 (0.35)	0.22+0.03 (0.27)
Pancreas	0.30±0.01	0.27±0.02 (0.89)	0.24±0.02 (0.81)
Spleen	0.42±0.03	0.27±0.02 (0.65)	0.24±0.03 (0.57)
Small intestine	1.61±0.24	0.73±0.25 (0.46)	0.30±0.05 (0.19)
Kidney	4.98±1.16	0.53±0.32 (0.11)	0.65±0.10 (0.13)
Muscle	0.25±0.16	0.19±0.02 (0.78)	0.19±0.05 (0.75)

*Mean± s.d. (ratio for the control) of 4 to 5 mice

**Two groups of mice were injected with 6-^{18}FFuc together with 5 mg and 50 mg

Table 3. Effects of L-fucose loading on percentages of the acid-insoluble radioactivity in FM3A tumor, liver and plasma 60 min after injection of 6-[^{18}F]fluoro-L-fucose.

	Control	L-Fucose loading groups	
		5 mg	50 mg
	(%)	(%)	(%)
FM3A	69.7±2.8	8.6±4.3	5.6±0.2
Liver	30.4±1.2	6.2±3.9	3.3±1.8
Plasma	55.1±3.2	4.2±2.3	2.1±2.3

Data present a mean ± s.d. (n=3~5)

Table 4. The tumor-to-liver ratios of radioactivity in whole body sections of tumor-bearing mice 60 min after injection of 6-[^{18}F]fluoro-L-fucose and [1-^{14}C]-L-fucose.

	Tumor-to-Liver Ratio*		
	3LL on the back	3LL in the lung	Spontaneous
	(n=6)	(n=3)	(n=2)
6-[^{18}F]Fluoro-L-fucose	1.46±0.63	2.10±0.59	2.08
[1-^{14}C]-L-Fucose	0.43±0.13	1.05±0.32	2.47

*The density of tumor and liver regions was measured, and the ratios in the individual mice were calculated. Data present a mean ± s.d.

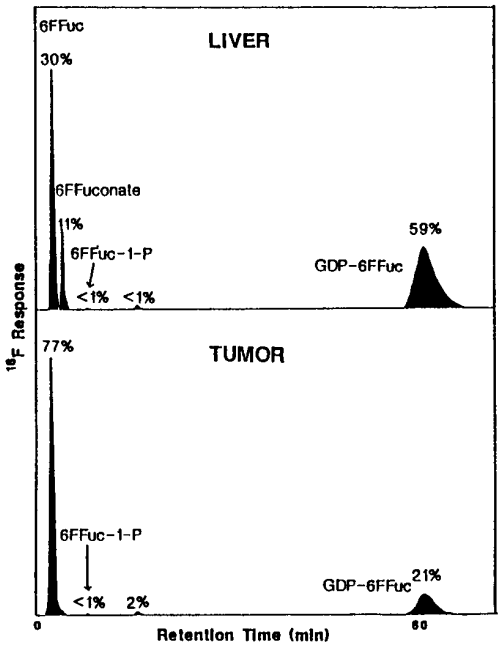

Fig . 1 High-performance liquid chromatograms of labeled metabolites.HPLC conditions: column, Radial-Pak SAX; eluent, 0.1 M sodium acetate, pH 4.1, containing 0.1 MNaCl; flow rate, 2 mL/min.

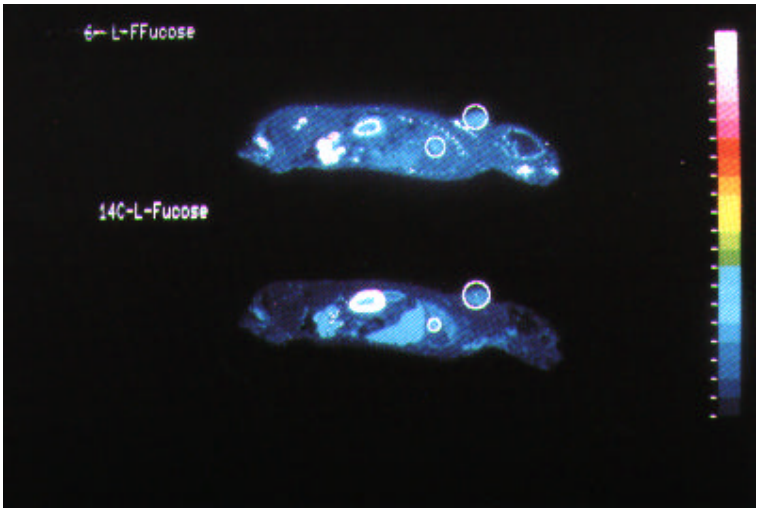

Fig 2. Autoradiograms of a 3LL-bearing mouse given with 6-^{18}FFuc (A) and ^{14}C-Fuc (B). A mouse injected with 37 MBq of 6-^{18}FFuc and 76 kBq of ^{14}C-Fuc simultaneously, and sacrificed 60 min after injection. The mouse was frozen and cut into 30 μm-thick slices. Respective autoradiograms of ^{18}F and ^{14}C were made.[3)]

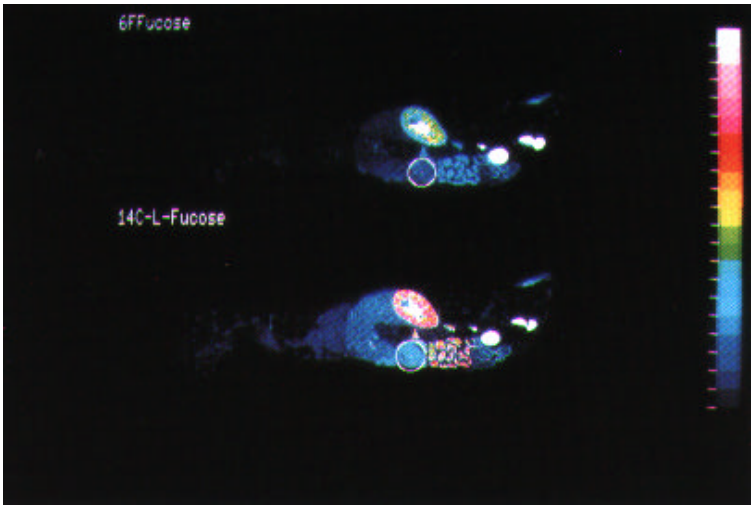

Fig 3. Autoradiograms of C3H/He mouse with spontaneous liver tumors injected with ^{18}FFuc (A) and ^{14}C-Fuc (B).

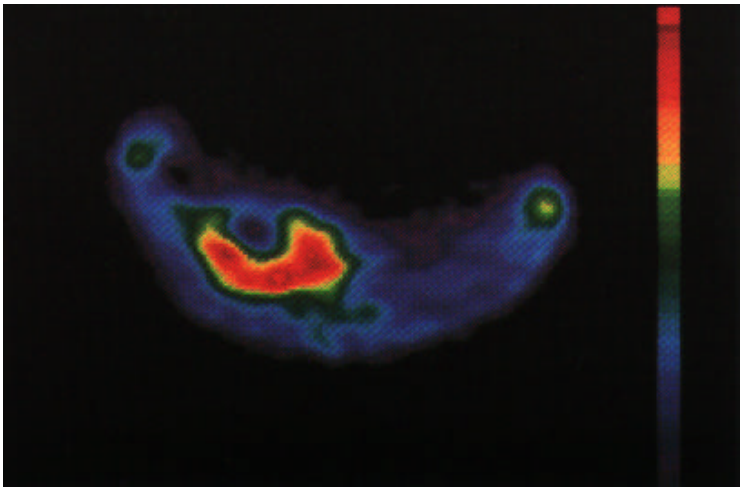

Fig 4. Positron emission tomographic image in rabbit hind legs region at 50-60 min after injection of 6-^{18}FFuc.