Tumor Uptake Study of 18F-labeled N-Acetylneuraminic Acids

III. 5. Tumor Uptake Study of ^{18}F-labeled *N*-Acetylneuraminic Acids

Ishiwata K., Ido T., Nakajima T., Ohrui H.*, Kijima-Suda I.** and Itoh M.***

Division of Radiopharmaceutical Chemistry, Cyclotron and Radioisotope Center, and Department of Food Chemistry, Faculty of Agriculture, Tohoku University, Central Research Institute, Mect Corporation***

Introduction

In recent years our group has proposed several positron-emitting tracers for assessing metabolic functions of tumors with positron emission tomography. Tumor accumulation of the tracers is based on enhanced metabolic activities. Recently, we focus our attention on the glycoconjugate synthesis in plasma membrane of tumors. Fucose and *N*-acetylneuraminic acid are constituents of glycoproteins and glycolipids in the plasma membrane. In previous papers, a potential of ^{18}F-labeled fucose analogues for tumoremetabolic imaging were investigated.[1,2] In this paper we report on tumor uptake studies of *N*-acetyl-3-[^{18}F]fluoroneuraminic acid (3-^{18}F-Neu5Ac) and *N*-acetyl-2-deoxy-2,3-di[^{18}F]fluoroneuraminic acid (2,3-di^{18}F-Neu5Ac). The former is a competitive inhibitor of sialidase, and the latter shows no effect on the sialidase.[3] 3-^{18}F-Neu5Ac is also a strong inhibitor of *N*-acetylneuraminic acid aldolase.[4]

Materials and Methods

3-^{18}F-Neu5Ac and 2,3-di^{18}F-Neu5Ac were prepared by the nucleophilic addition of $CH_3COO[^{18}F]F$ and $[^{18}F]F_2$, respectively, into methyl 5-acetamide-2,6-anhydro-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-D-glycero-D-galacto-2-enonate followed by the HPLC separation and alkaline hydrolysis.[5]

For tissue distribution study of the tracers tumor, models used were Donryu rats bearing AH109A hepatoma and Yoshida sarcoma (YS), Wistar rats bearing KEG-1 glioma, C3H/He mice with FM3A mammary carcinoma, BDF_1 mice with L1210 lymphoid leukemia, C57L/6 mice with Lewis lung carcinoma (3LL) or B16 melanoma. The tissue

uptake was expressed as the differential absorption ratio (DAR), i.e., (counts of tissue/total injected counts) × (g body weight/g tissue), to correct the data for body weight of animals.

FM3A, liver and kidney tissues and plasma samples of C3H/He mice sacrificed 120 min after injection of 3-^{18}F-Neu5Ac, were homogenized in 0.1 M $HClO_4$ and were divided to the acid-soluble and acid-insoluble fractions. The tissues were also homogenized in ethanol, and the metabolites in the ethanol-soluble fraction was analyzed by HPLC.

Results and Discussion

Two tracers showed very similar tissue distribution patterns (Tables 1 and 2). Radioactivity decreased rapidly with time in all tissues. In the liver the clearance of radioactivity was relatively slow. The radioactivity in blood were higher than in other tissues for 30 min. The highest uptake in the kidney represents the rapid excretion of both tracers into urine. For the first 10 min more than 40% of the radioactivity was measured into urine. Radioactivity levels in FM3A tumor were also decreased for 2 h. The largest tumor uptake ratios were found for the brain, but the ratios decreased with time. Only tumor-to-blood ratios were increased. Using seven tumor models the tumor uptake and tumor uptake ratio of two tracers were compared 30 min after injection (Table 3). At this time tumor uptake was very different among seven tumors. However, the respective DAR values for each of tracers in the same tumors were similar except for the L1210. On the other hand, the difference in tumor-to-organ ratios among each of tumors was relatively small, and the ratios were not necessarily parallel to the DAR values. When compared to two tracers, large different ratios were not observed for other organs in all tumor models although tumor-to-liver ratios for the 2,3-di^{18}F-Neu5Ac were smaller than those for 3-^{18}F-Neu5Ac. Because both tracers disappeared rapidly from many tissues, the altered metabolism in tumor-bearing animals, especially in excretion functions, effects on the DAR values and uptake ratios for each of tumors.

The metabolites of 3-^{18}F-Neu5Ac in the FM3A, liver, kidney and plasma were investigated in FM3A-bearing mice. Incorporation of the tracer into the acid-insoluble macromolecules was substantially inhibited. In the ethanol-soluble fraction three radioactive peaks were detected by HPLC analysis (Table 4). The third component was more acidic than 3-^{18}F-Neu5Ac and was stable in acidic conditions. The metabolite is not a CMP derivative, but may be 3-^{18}F-fluoropyruvic acid produced by *N*-acetylneuraminic acid aldolase.

In conclusion two ^{18}F-labeled Neu5Ac compounds with and without a competitive inhibitory action for a sialidase showed similar tissue distributions, and were rapidly cleared

from tumor and normal tissues. No significant different tumor uptake of two tracers was found in seven animal models. The tracers are not suitable for tumor imaging in vivo.

References

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Table 1. Tissue distribution of radioactivity after i.v. injection of N-acetyl-3-[^{18}F]fluoroneuraminic acid in FM3A-bearing mice.

	Uptake, DAR* Tumor-to-tissue Ratio 30 min (n=5)	60 min (n=5)	120 min (n=5)
FM3A	0.26±0.06 1.00	0.13±0.04 1.00	0.10±0.02 1.00
Blood	0.42±0.05 0.60	0.12±0.03 1.12	0.04±0.02 2.65
Brain	0.01±0.00 13.79	0.01±0.00 10.16	0.01±0.00 7.99
Heart	0.25±0.07 1.03	0.12±0.03 1.08	0.10±0.04 0.95
Lung	0.46±0.08 0.56	0.17±0.06 0.75	0.17±0.09 0.57
Liver	0.29±0.06 0.90	0.20±0.04 0.66	0.18±0.06 0.55
Pancreas	0.21±0.08 1.21	0.16±0.11 0.79	0.15±0.08 0.67
Spleen	0.17±0.08 1.50	0.08±0.03 1.55	0.05±0.01 1.84
Small intestine	0.34±0.09 0.76	0.18±0.05 0.73	0.29±0.17 0.34
Kidney	2.15±0.45 0.11	1.41±0.77 0.09	1.33±0.61 0.07
Muscle	0.13±0.03 1.98	0.09±0.04 1.46	0.05±0.02 2.09

*Values are mean ±s.d.

Table 2. Tissue distribution of radioactivity after i.v. injection of N-acetyl-2,3-di [^{18}F]fluoroneuraminic acid in FM3A-bearing mice.

	Uptake, DAR* Tumor-to-tissue Ratio		
	30 min (n=5)	60 min (n=4)	120 min (n=4)
FM3A	0.24±0.03 1.00	0.10±0.04 1.00	0.07±0.01 1.00
Blood	0.31±0.10 0.76	0.12±0.06 0.82	0.05±0.01 1.51
Brain	0.05±0.01 5.11	0.03±0.01 3.71	0.02±0.00 3.17
Heart	0.25±0.02 5.11	0.11±0.05 3.71	0.10±0.02 3.17
Lung	0.30±0.09 0.80	0.21±0.11 0.46	0.10±0.02 0.57
Liver	0.40±0.04 0.59	0.49±0.10 0.19	0.40±0.04 0.18
Pancreas	0.11±0.03 1.53	0.12±0.03 0.77	0.08±0.03 0.67
Spleen	0.09±0.01 2.55	0.15±0.02 0.63	0.06±0.01 1.18
Small intestine	0.28±0.08 0.84	0.21±0.02 0.05	0.11±0.03 0.08
Kidney	2.71±1.11 1.91	1.77±0.29 3.71	0.92±0.43 3.17
Muscle	0.12±0.03 1.91	0.03±0.01 3.71	0.02±0.00 3.17

*Values are mean ±s.d.

Table 3. Tissue distribution and tumor-to-organ ratio of radioactivity at 30 min after i.v. injection of N-acetyl-3-[^{18}F]fluoroneuraminic acid (3-^{18}F-Neu5Ac) or N-acetyl-2,3-di[^{18}F]fluoroneuraminic acid (2,3-di^{18}F-Neu5Ac) in tumor-bearing mice and rats.

		Uptake*	Tumor-to-organ Ratio**				
		DAR (n)	Blood	Brain	Lung	Liver	Muscle
3-^{18}F-Neu5Ac	FM3A	0.26±0.06 (5)	0.60	13.79	0.56	0.90	1.98
	L1210	0.22±0.04 (5)	0.85	11.05	1.33	1.57	2.58
	B16	0.15±0.03 (8)	0.77	11.22	1.09	0.91	2.73
	3LL	0.10±0.02 (5)	0.34	8.00	0.44	0.57	1.68
	AH109A	0.30±0.06 (4)	0.55	7.01	0.77	1.48	2.03
	KEG1	0.36±0.05 (3)	0.46	9.30	0.61	1.33	2.01
	YS	0.40±0.05 (5)	0.77	10.50	0.93	2.01	2.87
2,3-di^{18}F-Neu5Ac	FM3A	0.24±0.03 (5)	0.76	5.11	0.80	0.59	1.91
	L1210	0.45±0.18 (5)	0.69	14.25	1.02	0.64	2.50
	B16	0.13±0.05 (5)	0.97	8.54	1.02	0.31	3.04
	3LL	0.10±0.07 (4)	0.90	9.19	0.76	0.27	2.75
	AH109A	0.31±0.12 (5)	0.49	6.65	0.75	0.44	2.01
	KEG1	0.28±0.11 (5)	0.46	6.64	0.54	0.39	2.04
	YS	0.43±0.04 (5)	0.77	13.31	1.18	0.70	3.61

*Values are mean±s.d. **Values are mean calculated in each of seven animal models.

Table 4. Percentages of metabolites in the ethanol-soluble fraction of FM3A-bearing mice 120 min after injection of N-acetyl-3-[^{18}F]fluoroneuraminic acid.

	Peak 1 (4.5 min)*	Peak 2 (6.5 min)*	Peak 3 (12.5 min)*
FM3A		99.9%	0.1%
Liver	2.7%	92.0%	5.3%
Kidney	3.2%	92.5%	4.3%
Plasma		100%	

A Radial-Pak SAX column was used with 0.1M NaCl-containing 0.1 M sodium acetate,pH 4.1 at a flow rate of 2.0 mL/min.
*Retention time.
Peak 2 represents N-acetyl-3-[^{18}F]fluoroneuraminic acid.