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Metabolism of 2-deoxy-2-[^{18}F]fluoro-D-glucose: Presence of 2-deoxy-2-[^{18}F]fluoro-D-glucose 6-phosphate in plasma of mice, rats and humans

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INTRODUCTION

FLUORINE-18 labeled 2-deoxy-2-fluoro-D-glucose ([^{18}F]FDG) is used for *in vivo* measurement of cerebral glucose utilization with positron emission tomography (PET). Kinetic analysis using the [^{18}F]-FDG is based on the deoxyglucose method developed by Sokoloff et al.[1] In this model the trapping of 2-deoxy-D-glucose 6-phosphate in the brain and no release of the phosphate derivative from the brain into plasma are essential. Investigators have checked this essential premise in cats, in which they measured the ^{14}C levels in arterial and cerebral venous blood and confirmed no release of 2-deoxy-D-[^{14}C]glucose 6-phosphate from the brain. Because FDG has similar biochemical properties to 2-deoxy-D-glucose, [^{18}F]FDG has been applied to PET studies.[2,3] In mice the metabolic trapping by 2-deoxy-2-[^{18}F]fluoro-D-glucose 6-phosphate ([^{18}F]FDG-6-P) has been examined in several tissues.[4] However, the presence of [^{18}F]FDG-6-P in plasma has not been studied. Recently, in the studies on metabolism of [^{18}F]FDG in experimental tumors, we found the presence of [^{18}F]FDG-6-P in the plasma. In this paper we report on the presence of [^{18}F]FDG-6-P in the plasma of mice, rats and humans. Also the influence of the [^{18}F]FDG-6-P in the plasma is evaluated by kinetic analysis.

MATERIALS AND METHODS

Synthesis of [^{18}F]FDG and [^{18}F]FDG-6-P

[^{18}F]FDG was synthesized by the reaction of [^{18}F]-CH_3COOF with 3,4,6-tri-0-acetyl-D-glucal in Freon-11.[5]

[^{18}F]FDG-6-P was synthesized enzymatically by the method of Bessell and Thomas.[6] In a final volume of 0.1 m*l* the following components were incubated for 1 hour at 37°C at the specified concentrations: 0.1 M Tricine-NaOH, pH 8.2, 0.1 M $MgCl_2$, 60 mM ATP, 1 mCi/m*l* [^{18}F]FDG and 56 units/m*l* yeast hexokinase (Boehringer, Mannheim GmbH).

Metabolic studies

As experimental animals two kinds of mice, C3H/He and ddY mice, and two kinds of rats, Wistar and Donryu rats, were used. In a group of Donryu rats, ascitic hepatoma, AH109A, was implanted as described previously.[7]

Mice were injected with 1.0 to 1.5 mCi of [^{18}F]-FDG through a lateral tail vein. The mice were sacrificed by cervical dislocation at 60 min after the injection. Blood was removed by heart-puncturing using a syringe, precoated with heparin. The blood was centrifuged at 2,500 rpm for 5 min to collect plasma. Rats anesthetized with sodium pentobarbital were injected with 4.6 to 6.0 mCi of [^{18}F]FDG through a cannula in a femoral vein. Blood was collected through a cannula in a femoral artery at 10, 30 and 60 min after the injection. Plasma was obtained as described above. Brain and tumor tissues were collected.

In human studies 5.0 to 8.0 mCi of [^{18}F]FDG was injected intravenously. At 60 min after the injection, blood was collected from an artery and plasma was obtained as described above.

Plasma was treated with an equivalent volume of 0.4 M $HClO_4$ at 0°C and subsequently centrifuged at 2,500 rpm for 5 min. The precipitate was washed

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with 0.5 m*l* of 0.2 M $HClO_4$ and centrifuged as described above. The acid-soluble fractions were combined. The brain and tumor tissues were homogenized in 0.3 M $HClO_4$. The acid volume was about two times the equivalent volume of the tissue. The homogenate was centrifuged at 2,500 rpm for 5 min. The supernatant was collected and neutralized with 1 M KOH. The solution was kept at ice-cold temperature and precipitated $KClO_4$ was removed by membrane filtration.

Metabolites in the rat plasma were treated with phosphatase. The mixture containing 0.88 m*l* plasma, 0.1 m*l* 1 M glycine-KOH, pH 10.5, 10 μl 0.1 m $MgCl_2$, 10 μl 10 mM $ZnCl_2$ and 1 mg alkaline phosphatase (calf intestine, Miles Laboratories) was incubated at 37°C for 60 min. The $HClO_4$-soluble fraction was obtained as described above.

Metabolites of [^{18}F]FDG in the plasma and tissue samples were analyzed by two different HPLC systems. For measuring [^{18}F]FDG, the acid-soluble sample was applied on an Aminex HPX-87C column (7.8 mm ID × 300 mm length, Bio-Rad), which was used at 85°C with H_2O at a flow rate of 0.8 m*l*/min. For measuring [^{18}F]FDG-6-P, Radial-PAK SAX (8 mm ID × 100 mm length, Waters) was used at room temperature with 0.10 M CH_3COOH-CH_3COONa, pH 4.1, containing 0.15 M NaCl at a flow rate of 2.0 m*l*/min. The plasma sample was directly applied to this column without the acid treatment.

RESULTS AND DISCUSSION

Figure 1 shows HPLC profiles of [^{18}F]FDG preparation and metabolites in the plasma sample of C3H/He mice. The authentic [^{18}F]FDG was eluted as peak *b* on a cation exchange column, Aminex HPX-87C, and as peak *c* on an anion exchange column, Radial-PAK SAX. In the [^{18}F]FDG preparation, no ^{18}F was detected in the regions of peaks *a* and *d*.

In the analysis of metabolites in the plasma, peaks *a* and *d* on the Aminex HPX-87C column and the Radial-PAK SAX column, respectively, have the same retention time as enzymatically synthesized [^{18}F]FDG-6-P. After treatment of the plasma with phosphatase, these peaks disappeared. In the rat brain and AH109A tumor samples, the peaks *a* and *d*, were for the main metabolite. From these results the peaks *a* and *d* were identified to be [^{18}F]FDG-6-P on the two HPLC columns.

In the plasma sample [^{18}F]FDG was detected in the peaks *b* and *c* (Fig. 1B). Because the peak *c* has no retention time, it is possible that the peak contains other neutral and/or cationic components. The percentage of the [^{18}F]FDG was determined by analysis on an Aminex HPX-87C column. Similarly, the peak

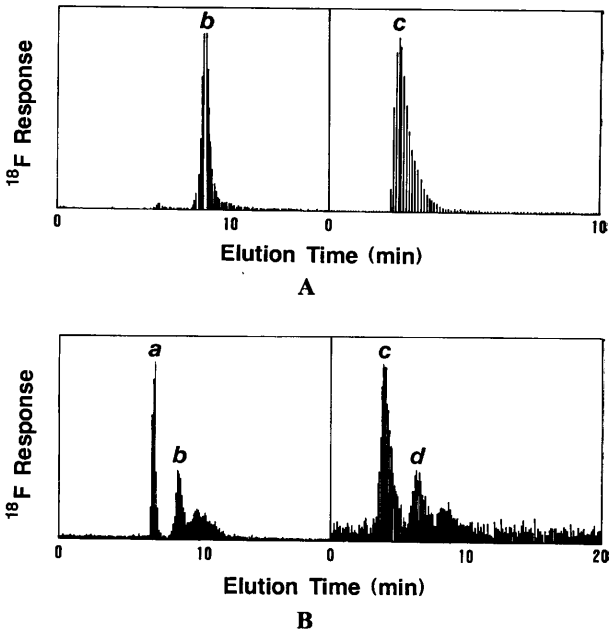

Fig. 1 Analytical profiles of [^{18}F]FDG preparation and metabolites in the plasma sample of C3H/He mice. Fig. 1A represents the [^{18}F]FDG and Fig. 1B shows the plasma sample. The left figures, respectively, show the results on the Aminex HPX-87C column and the right figures, respectively, show the results on the Radial-PAK SAX column.

d on an anion exchange column was calculated as the percentage of [^{18}F]FDG-6-P. There are other radioactive materials besides [^{18}F]FDG and [^{18}F]-FDG-6-P in the plasma sample of C3H/He mice. It is not certain whether these are real metabolites of [^{18}F]FDG or false metabolites derived from impurities in the [^{18}F]FDG preparation. Similar radioactive materials were detected in the plasma of ddY mice. However, in plasma samples of rats and humans only two peaks of [^{18}F]FDG and [^{18}F]FDG-6-P were observed.

In Table 1 the analytical results in two kinds of mice, in two kinds of rats, and in humans are presented. In mice, the percentages of [^{18}F]FDG-6-P in the plasma were considerably high, especially in C3H/He mice, in which the percentage of [^{18}F]FDG-6-P was larger than that of [^{18}F]FDG. In both kinds of rats the amount of the [^{18}F]FDG-6-P was much less than that in mice. It is confirmed that the percentage of [^{18}F]FDG-6-P increased with time, and the value in the Donryu rats was slightly larger than that in the Wistar rats. These significant differences between mice and rats may be dependent on animal species. It is also possible that the different methods to collect blood may influence the results. Compared to the results in mice and rats the percentage of [^{18}F]FDG-6-P in human plasma was very low.

Table 1 Percentage of 2-deoxy-2-[^{18}F]fluoro-D-glucose 6-phosphate ([^{18}F]FDG-6-P) in plasma of mice, rats and humans after intravenous injection of 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG)

		Time (min)	Number	[^{18}F]FDG-6-P (%)	[^{18}F]FDG (%)
ddY Mouse	Plasma	60	3	36.1±2.2	43.2±1.0
C3H/He Mouse	Plasma	60	3	45.4±12.5	31.1±10.2
Wistar Rat	Plasma	10	3	3.4±1.3	95.8±2.1
		30	3	4.4±3.0	94.5±2.1
		60	3	8.4±3.4	91.3±3.4
Donryu Rat	Plasma	10	3	3.6±0.8	95.2±0.7
		30	3	5.8±1.0	91.7±0.8
		60	3	12.0±0.2	87.8±3.2
Tumor-bearing	Brain	60	3	97.4±1.0	
Donryu Rat	AH109A	60	3	98.1±3.6	
Human	Plasma	60	4	3.0±1.6	

The percentages of [^{18}F]FDG and [^{18}F]FDG-6-P were determined by HPLC on an Aminex HPX-87C column and a Radial-PAK SAX column, respectively. Radiochemical purity of [^{18}F]FDG used in these animal experiments was 97.9±1.0 (n=5). In the analysis of [^{18}F]FDG preparations on the Radial-PAK SAX column no ^{18}F was detected in the elution time of [^{18}F]FDG-6-P.

Although the percentage of [^{18}F]FDG-6-P increased with time in rats, the concentration of [^{18}F]FDG-6-P is nearly constant from 10 min to 60 min. Preliminarily, it was observed that the enzymatically synthesized [^{18}F]FDG-6-P was converted slowly to [^{18}F]FDG when it was incubated with the rat plasma. On the other hand, [^{18}F]FDG was not phosphorylated *in vitro* in the mouse or rat plasma. These results suggest that after phosphorylation of [^{18}F]FDG by hexokinase in tissues, a small amount of [^{18}F]FDG-6-P is released into the plasma, in which some of the [^{18}F]FDG-6-P is dephosphorylated by phosphatase, and consequently a low level of the [^{18}F]FDG-6-P is observed in rats. This possibility together with species differences may explain why there was no phosphate derivative of ^{14}C-labeled deoxyglucose in cats.[1] It is also possible that the biochemical properties of FDG are slightly different from those of deoxyglucose. In this study we did not investigate which organ is responsible for the presence of [^{18}F]FDG-6-P in the plasma and whether the [^{18}F]FDG-6-P is released from the brain tissue into plasma.

For measurement of the cerebral metabolic rate of glucose (CMRGlc) using [^{18}F]FDG by the deoxyglucose method,[1] the presence of [^{18}F]FDG-6-P in the plasma alters the input function of glucose. When the concentration of [^{18}F]FDG in the plasma is not corrected for the presence of the [^{18}F]FDG-6-P, the CMRGlc in the Wistar rat is calculated to be 97% of the corrected value by the autoradiographic method of Kameyama et al.[8] In the Donryu rat the corresponding figure is 94%. In human studies with PET, the presence of [^{18}F]FDG-6-P does not influence substantially the measurement of CMRGlc. The error based on the presence of [^{18}F]FDG-6-P in human studies is less than 1% with a hypothesis that the level of [^{18}F]FDG-6-P is constant from 10 min to 60 min as observed in rats.

In the brain sample more than 90% of the ^{18}F was detected as the [^{18}F]FDG-6-P at 60 min described in the previous reports.[4] Recently, nucleotide derivative was detected as a minor metabolite of [^{18}F]FDG in rat tumor[9] and tissue cultures.[10,11] However, we could not detect any other metabolites of [^{18}F]FDG besides [^{18}F]FDG-6-P in AH109A tumor.

In conclusion, the presence of [^{18}F]FDG-6-P in the plasma was observed in mice, rats and humans after intravenous injection of [^{18}F]FDG. In the experimental studies using [^{18}F]FDG in rats the cerebral glucose utilization is calculated to be larger in the presence of the [^{18}F]FDG-6-P compared to the experiments without correction. However, its influence is negligible in human studies with PET.

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