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THE SLOW METABOLISM OF L-[2-^{18}F]-FLUOROPHENYLALANINE IN RAT

M.Murakami, K.Takahashi, Y.Kondo*, S.Mizusawa†, H.Nakamichi†, H.Sasaki, E.Hagami, H.Iida, I.Kanno, S.Miura, I.Itoh, and K.Uemura

Departments of Radiology and Nuclear Medicine, Neurology*, Internal Medicine†, Research Institute for Brain and Blood Vessel-Akita, 6-10 Senshu-Kubota-Machi, Akita City, Akita 010, JAPAN

SUMMARY

The stability of L-[2-^{18}F]-fluorophenylalanine (2-[^{18}F]-Phe) was compared with that of natural amino acid, L-[2,6-^{3}H]-Phenylalanine (2,6-[^{3}H]-Phe). The mixture of 2-[^{18}F]- and 2,6-[^{3}H]-Phe was injected to Wistar rats. The separated arterial plasma was followed by acid treatment and chromatographical analysis. The plasma [^{18}F]- and [^{3}H]-radioactivities decreased with the time and showed the lowest value at 20 min after injection. After that, [^{3}H]-radioactivity increased significantly up to 60 min, while [^{18}F]-radioactivity remained at the lowest level. The [^{3}H]- and [^{18}F]-radioactive macromolecules appeared in the plasma at 10 and 20 min, respectively. In the acid soluble fractions of the plasma and the brain at 60 min, more than 80 % of [^{18}F]-radioactivity existed as 2-[^{18}F]-Phe, while 2,6-[^{3}H]-Phe was less than 15 %. These data suggest the slow metabolism of 2-[^{18}F]-Phe as compared with that of natural Phe. In conclusion, 2-[^{18}F]-Phe is suitable to the compartment analysis using positron emission tomography especially at the process of amino acid transport to the brain due to its stability for the period necessary to kinetic analysis.

Key words: 2-[^{18}F]-Phe, 2,6-[^{3}H]-Phe, metabolic speed, rat

INTRODUCTION

Amino acid tracers labelled with positron emitting radionuclides have become important in in-vivo human study to measure

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transport across the blood-brain-barrier and fixation into macromolecules with various pathological conditions. The quantitative analysis of the above physiological and biochemical behaviours requires that the tracer is stable during the measurement period or that its metabolic compartments are simple even if the metabolism occurs. At present, natural amino acids, L-[1-^{11}C]-leucine and L-[methyl-^{11}C]-methionine, are widely used in clinical studies. However, their radioactive carbons may be incorporated into various biomolecules, thus resulting in metabolic compartments which require complex kinetic analysis. The artificial amino acids such as [1-^{11}C]-aminocyclopentacarbonic acid and [1-^{11}C]-aminocyclohexacarbonic acid, may not reflect the behaviour of the natural amino acids because of the entirely different structures of the molecules. On the other hand, the fluorination of natural amino acid is thought to have the following advantages. The fluorinated amino acid may behave as the natural amino acid because its structure is close to that of the natural type. The metabolism may be delayed because fluorine shows strong electronegativity. From this standpoint, three [^{18}F]-fluorophenylalanine isomers and their biodistribution in animals were studied [1], and D,L- [4-^{18}F]-fluorophenylalanine was applied to human pancreas imaging [2]. However, since they observed only the accumulation of the radioactivity, the chemical forms of the tracers in the blood and tissue for quantitative compartment analysis using positron emission tomography have not been clarified.

We synthesized L-[2-^{18}F]-fluorophenylalanine (2-[^{18}F]-Phe) and L-[3-^{18}F]-fluorotyrosine [3]. In the case of 2-[^{18}F]-Phe, almost all the acid-soluble radioactivity was contained in the amino acid fraction in rat brain [3]. However, the chemical form of the radioactive amino acid was unclear, whether 2-[^{18}F]-Phe or another [^{18}F]-amino acid. This paper compares 2-[^{18}F]-

Phe and 2,6-[^{3}H]-Phe to clarify the chemical form of acid-soluble radioactivity and assesses their metabolic speeds.

MATERIALS AND METHODS

Tracer

2-[^{18}F]-Phe was synthesized by the electrophilic substitution reaction. The target gas, neon containing 0.5 % carrier F_2, was filled in 141 ml chamber up to 3.0 Kg/cm^2 and was irradiated with 6.8 MeV deuterons. After the end of the irradiation, [^{18}F]-F_2 was recovered through AcOK/AcOH column into the vessel containing 100 μ mole of L-phenylalanine in 10 ml of CF_3COOH at 0°C. At the end of bubbling, CF_3COOH was evaporated off under vacuum. The [^{18}F]-radioactive residue was dissolved in small amount of H_2O and was injected to the preparative HPLC (Waters, μBondapak C-18, 19 × 150 mm, 90 % water, 10 % CH_3OH with 0.1 % AcOH as a solvent). The 2-[^{18}F]-Phe fraction was collected, evaporated up, dissolved with one ml of saline, and passed through 0.22 μm pore filter. The specific activity at this point of time was around 300 mCi/m mole. A portion of above 2-[^{18}F]-Phe solution (equivalent to 1.2 – 1.8 mg of 2-F-Phe, 2-3 mCi) was mixed with 20 μCi of 2,6-[^{3}H]-Phe (NEN, specific activity; 60 Ci/m mole). This mixture of the two tracers was the dose per animal.

Analytical procedures

Male Wistar rats weighing from 200 to 300 g were used after 24 hrs fasting. The value of blood glucose at the experiment was 129 ± 21 mg/dl (mean ± SD, N=6). The tracer was injected at conscious condition from the polyethylene cannule inserted into the internal jugular vein. To examine the time course of plasma radioactivity, about 100 μl of arterial blood was sampled at 5, 10, 20, 30, 45, 60 and 120 min after tracer injection from the

cannule placed in the femoral artery. After the centrifugation, a portion of the plasma was removed for the measurement of the differential absorption ratio (DAR). DAR was represented as (counts/g tissue) × (g body weight/total injected counts). The remaining plasma was weighed, added the 1.0 ml of 5 % perchloric acid, centrifuged and the acid-soluble layer was separated. This acid treating process was repeated twice, and the combined acid-soluble layer was measured for its radioactivity.

For the chromatographical study of the acid-soluble radioactivity in the plasma and the brain, the blood was sampled by the heart puncture at 60 min after the tracer injection and the rat was sacrificed by K^+ injection. A portion of the plasma and the cerebrum were removed for DAR measurement, and about 300 mg each of samples were treated as mentioned above, and acid-soluble fractions were obtained. The HPLC analysis using reverse-phase column (Waters, μBondapak C-18, 7.6 × 300 mm, 90 % water, 10 % CH_3OH with 0.1 % AcOH) was carried out after the removal of $KClO_4$ by the neutralization with K_2CO_3. In the case of gel-filtration chromatography (Toyo-Soda, TSK-3000 SW, 7.5 × 600mm, 0.2 M-KH_2PO_4, pH 6.8), the plasma was injected to HPLC without any pretreatment.

The plasma, the cerebrum tissue, the acid-soluble fractions and the fractions of HPLC eluates were measured for their [^{18}F]-radioactivity with well-type gamma counter. The samples were transferred to the liquid-scintillation counting vial, and were measured their [^{3}H]-radioactivity with liquid scintillation counter (Beckman 9800) after the adjustment of water content, solbilization with Protosol®(NEN), decolorization with H_2O_2, neutralization with HCl and mixing with 12 ml of liquid scintillator (Amersham, ACS-II). There were at least three days interval between [^{18}F]-and [^{3}H]-measurements. The percent of acid-insoluble fraction was calculated from the tissue radio-

activity and its ratio of acid-soluble fraction. All the data were expressed as the mean ± SD of three rats.

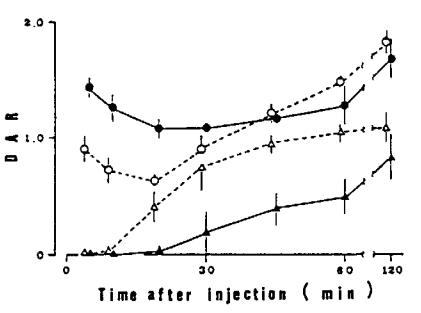

Fig. 1

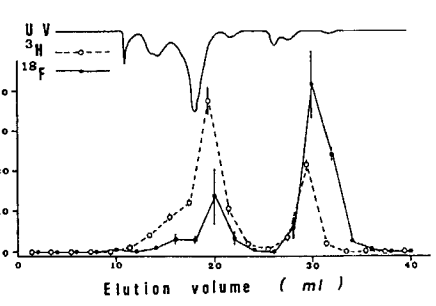

Fig. 2

Fig. 1

The time courses of the radioactivity in the arterial plasma. The solid line with closed circles and the dotted line with open circles represent the differential absorption ratio of [^{18}F]- and [^{3}H]-radioactivity, respectively. The solid line with closed triangles and the dotted line with open triangles represent the percent fraction of the [^{18}F] and [^{3}H]-radioactive acid-insoluble macromolecules in the plasma, respectively.

Fig. 2

The gel-filtration chromatogram of the raw plasma at 60 min after tracers injection. The percentages of 2 ml fractions to the total radioactivity applied are represented. The chart of UV absoprtion is the one case of plasma samples.

RESULTS

The time courses of plasma [^{18}F]-and [^{3}H]-radioactivity are shown in Fig. 1. Both the radioactivities in the plasma showed the lowest values at 20 min after the tracer injection and increased gradually with the time. The increase of [^{3}H]-radioactivity was greater than that of [^{18}F]-radioactivity. In the case of [^{18}F], the appearance of radioactive macromolecules in the plasma was delayed as compared with [^{3}H], and the ratio of

[^{18}F]-radioactive macromolecules to the plasma was lower than that in the case of [^{3}H] at any observed period. At 60 min after tracer injection, plasma DAR (percent of acid-insoluble fraction) of [^{18}F]- and [^{3}H]-radioactivity was 1.28 ± 0.08 (27.6 ± 7.8) and 1.47 ± 0.04 (54.3 ± 3.6), respectively. At this time point, cerebral DAR (percent of acid-insoluble fraction) of [^{18}F]- and [^{3}H]-radioactivity was 0.74 ± 0.16 (39.6 ± 7.5) and 0.62 ± 0.16 (39.4 ± 13.7), respectively.

The gel-filtration chromatogram of the raw plasma at 60 min after injection is shown in Fig. 2. The radiochromatograms of [^{18}F]- and [^{3}H]-radioactivity had two peaks each, and the former peak had the same elution volume as that of the plasma protein. The latter peak corresponded to the low molecular acid-soluble fraction.

The acid-soluble fraction of the plasma and the cerebral tissue were analysed by the reverse-phase HPLC (Fig. 3A,B). At 60 min after the tracer injection, in the case of [^{18}F]-radioactivity, more than 80 % of acid-soluble radioactivity both

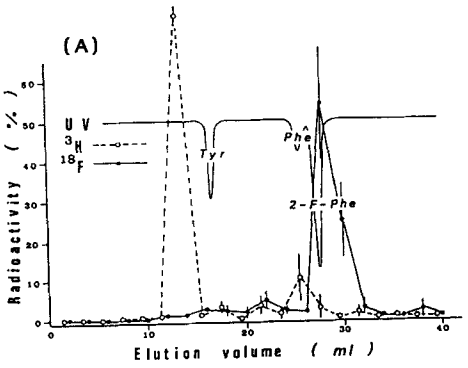

Fig. 3A

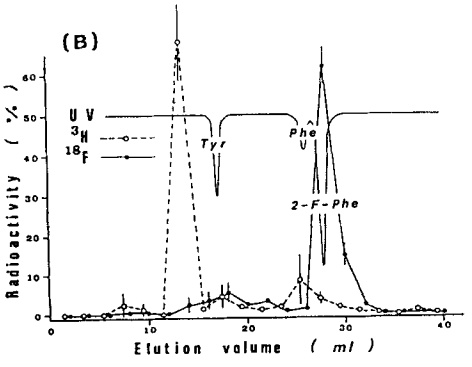

Fig. 3B

Fig. 3

The reverse-phase chromatogram of the acid-soluble fraction of the plasma (A) and the cerebrum (B) at 60 min after tracers injection. The percentages of 2 ml fractions to the total radioactivity applied are represented. The chart of UV absorption is the one case of authentic compounds added to the plasma or the cerebrum supernatant.

in the plasma and brain existed as 2-[^{18}F]-Phe. However, in the case of 2,6-[^{3}H]-Phe, the original chemical structure was less than 15 %.

DISCUSSION

The main metabolic pathways of the tracers are postulated according to the reports [3, 4] as shown in Fig. 4. The routes of the radioactivity distribution are similar between the tracers. So, the bioavailability and the metabolic speed of 2-[^{18}F]-Phe were compared to that of 2,6-[^{3}H]-Phe for quantitative kinetic analysis using positron emission tomography.

The appearance time of [^{18}F]-radioactive macromolecules into plasma was delayed from that of [^{3}H]-radioactive macromolecules (Fig. 1). These radioactive macromolecules corresponded to the plasma protein which was detected by UV absorption (Fig. 2). Furthermore, the percentage of [^{18}F]-macromolecules in the plasma was lower than that of [^{3}H]-macromolecules in all cases (Fig. 1,2). These findings show that the incorporating speed of 2-[^{18}F]-Phe into the plasma protein is slower than that of 2,6-[^{3}H]-Phe. Thus, the metabolic speed of 2-[^{18}F]-Phe is definitely slow. In above time-course study in plasma, around 700 μl of blood was withdrawn all together. However, we thank that there was no significant physiological change, because the volume was less than 5 % of the total blood. At 60 min after the tracer injection, more than 80 % of acid-soluble [^{18}F]-radioactivities both in the plasma and the brain existed as 2-[^{18}F]-Phe. However, in the case of 2,6-[^{3}H]-Phe, the original chemical structure was less than 15 % (Fig. 3A,B). The result also supports the slow metabolism of 2-[^{18}F]-Phe.

The time course concerning 2,6-[^{3}H]-Phe metabolite in the plasma was assessed in one rat. The [^{3}H]-radioactive metabolite

increased with the time and its percentage in the acid-soluble fraction at 5, 20 and 60 min was 18.7, 39.9 and 78.5 %, respectively. The elution volume of this [^{3}H] metabolite from the above reverse phase HPLC column agreed with that of fumaric acid, but not with p-hydroxyphenyl pyruvic acid and homogentisic acid. Thus, the main radioactive metabolite of 2,6-[^{3}H]-Phe was thought to exist as [^{3}H]-fumaric acid and other organic acid in the tricarbonic acid cycle, and also might be transported from the blood to the brain.

On the other hand, in the case of 2-[^{18}F]-Phe, it is reliable in spite of the single HPLC condition that the main radioactive peak in acid-soluble fraction is 2-[^{18}F]-Phe, because the amount of radioactive organic acid and catecholamines was negligible in the brain, and the elution of fluorinated tyrosine from the column was prior to that of phenylalanine as shown in our previous report [3]. Therefore, the metabolic speed of 2-[^{18}F]-Phe in-vivo may be very slow as compared with that of the natural amino acid.

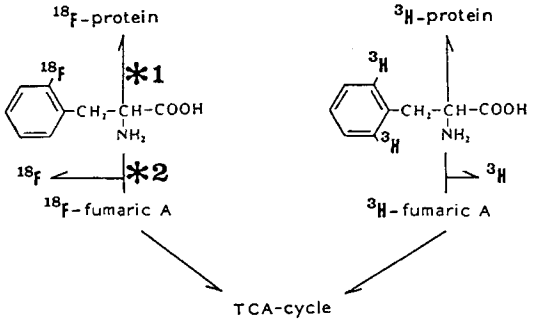

Fig. 4

Fig. 4

*The postulated metabolic pathways according to Ref. 3, 4. The degradative fate of radioactive atoms is whether the elimination from the tracers at the metabolic step in which homogentisic acid generates or the transfer into tricarbonic acid cycle via fumaric acid. *1 and *2 represent moderately and significantly slow, respectively, as compared with that in the case of 2,6-[^{3}H]-Phe.*

In this study cerebral and plasma DARs were almost the same between two tracers, and 2-[^{18}F]-Phe was incorporated into acid-insoluble macromolecules in the brain as same extent as 2,6-[^{3}H]-Phe did, in spite of its incorporating speed into plasma protein was slow. From the result, it was suggested that 2-[^{18}F]-Phe was transported to the brain by the same mechanism as that of natural amino acid. However, the transport process in this study was thought to be not physiological condition because very high dose of 2-[^{18}F]-Phe was injected. So, followings must be clarified for the availability of 2- [^{18}F]-Phe.

1) whether 2-[^{18}F]-Phe competes with 2,6-[^{3}H]-Phe or not.
2) whether cerebral DAR of 2-[^{18}F]-Phe is merely based on its physiochemical lipophilicity or not.

Above questions were discussed in another study (see Appendix). As a results, it was indicated that 2-[^{18}F]-Phe was transported not by mere diffusion but by the same carrier as that of 2,6-[^{3}H]-Phe. However, further studies concerning the carrier effects and the characteristics as a substrate for Phe-acyltRNA synthetase may be desired.

In conclusion, 2-[^{18}F]-Phe is suitable tracer, especially in the quantitative kinetic analysis for amino acid transportation using positron emission tomography because of its slow metabolism and simple metabolic pathway.

APPENDIX

Two groups were compared to evaluate whether 2-[^{18}F]-Phe distributed to the brain merely by its lipophilic characteristics or by carrier mediated transport system of amino acid (Table 1).

The fasted condition is well known to cause the increase of cerebral amino acid level. If 2-[^{18}F]-Phe distributes to the

Table 1 The effect of fasting state on amino acid distribution to the brain

Fasting (hrs)		24	72
Body weight (g)		235 ± 22	289 ± 5*
Blood glucose (mg/dl)		129 ± 21	126 ± 8
2-[^{18}F]-Phe (mg)		1.61 ± 0.11	1.30 ± 0.07*
DAR (cerebrum)	^{18}F	0.74 ± 0.16	1.05 ± 0.24*
	^{3}H	0.62 ± 0.16	0.83 ± 0.13*

** : $p < 0.05$ compared with 24 hours fasted group. All the data (mean ± SD; n=6) was obtained by the same procedure as shown in materials and method.*

brain by the simple diffusion mechanism because of its lipophilicity, cerebral DARs in two groups must be equal. As a result, in 72 hours fasted group, cerebral DAR of ^{18}F-radioactivity increased as well as that of ^{3}H-radioactivity, as compared with 24 hours fasted group, in spite of their heavy body weight and the small dose of 2-[^{18}F]-Phe.

Thus, carrier mediated transport of 2-[^{18}F]-Phe was evidently indicated.

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