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Preclinical study

Metabolic studies of [^{18}F-α-methyl]tyrosine in mice bearing colorectal carcinoma LS-180

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Brain and tumor uptake of [^{18}F-α-methyl]tyrosine (^{18}F-AMT) and the incorporation into each of four fractions (lipid, RNA, DNA and protein) were investigated in mice bearing LS180 colorectal carcinoma. Homogenized tissues were analyzed by the fractionation method into an acid-soluble fraction (ASF) and an acid-precipitable fraction (APF). The APF was further investigated to assess the incorporation of ^{18}F-AMT into each fraction. Incorporation into four fractions of brain and tumor at 60 min post-injection was 20 and 12%, respectively; 10% of the activity was incorporated to lipid in brain and 5% in tumor. There was 5, 2 and 2% incorporation with RNA, DNA and protein, respectively. Metabolites in ASF were analyzed by high-performance liquid chromatography and thin-layer chromatography. There was only one radioactive peak, which corresponded to ^{18}F-AMT. The incorporation of ^{18}F-AMT into lipid was twice that of ^{18}F-AMT in tumor. The uptake of ^{18}F-AMT in tissues was rapid and accomplished before 30 min, and then slowly diffused in blood. These results implied that ^{18}F-AMT was metabolized to protein to only a small extent and trapped as intact ^{18}F-AMT in cells up to 60 min. We conclude that ^{18}F-AMT is a promising tracer for tumor imaging and quantification of the transport rate using two-compartment models. [© 1999 Lippincott Williams & Wilkins.]

Key words: Colorectal carcinoma, [^{18}F-α-methyl]tyrosine, imaging, metabolism.

Introduction

Metabolic imaging of tumors by positron emission tomography (PET) is used in characterizing tumor growth in terms of increased protein synthesis and glucose metabolism. Several labeled compounds were developed for this purpose. Most of the compounds labeled with ^{11}C were natural amino acids, especially [^{11}C]leucine, [^{11}C]methionine and [^{11}C]tyrosine.[1-4] The drawback of these labeled compounds is in the short half-life which does not provide sufficient time to acquire a longer period of PET scanning. In addition, there are many metabolic pathways other than protein synthesis. Deehan *et al.*[5] used a longer half-life isotope (^{125}I) and investigated the potential use of radioiodinated α-methyl-tyrosine (^{125}I-AMT) in animal models. Langen *et al.*[6] used [^{124}I-iodo-α-methyl]tyrosine (^{124}I-AMT) to evaluate its kinetic behavior in brain and tumors using PET. These reports showed that I-AMT was highly accumulated in brain and tumor. The uptake in the tumor and brain using ^{123}I-AMT by SPECT was then evaluated. However, ^{123}I-AMT had high uptake in tumor but it was not incorporated into proteins.[7-9]

We have selected L-AMT, which is an inhibitor of tyrosine hydroxylase[10] and also has high uptake in tumors. The labeling of AMT with ^{18}F, which has a longer half-life compared to ^{11}C and therefore may have advantages over ^{11}C-labeled amino acids for the investigation of tissue with relative slow protein synthesis such as brain and for application in institutions with an offsite but nearby cyclotron, was performed using a previously reported remote controlled system.[11] We have conducted animal experiments in order to investigate ^{18}F-AMT for possible clinical use. The tumor uptake of ^{18}F-AMT at 60 min post-injection was significantly higher than that of [^{18}F]fluorodeoxyglucose (FDG) and equal to that of [^{11}C]methionine.[12,13] Although ^{18}F-AMT proved to be a very promising imaging agent as determined from our PET studies,[12,13] little information on the metabolism of ^{18}F-AMT is known. In this paper, we report the metabolism of ^{18}F-AMT in tumor and brain.

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Materials and methods

Preparation of radiopharmaceutical

^{18}F was produced from the deuteron bombardment of 0.1% F_2-neon mixture by the ^{20}Ne (d, α). ^{18}F nuclear reaction using the cyclotron facility at the Gunma University Hospital cyclotron center. F_2 gas was converted to ^{18}F-labeled acetylhypofluorite ($CH_3COO^{18}F$) by passage through a cartridge containing powdered NaOAC. Synthesis of [^{18}F-L-α-methyl]-tyrosine (^{18}F-AMT) was performed by direct electrophilic fluorination. The method of labeling and high-performance liquid chromatography (HPLC) purification of this radiopharmaceutical were previously reported.[11]

Preparation of tumor-bearing mice

Seven-week-old female BALB/c mice (18–22 g) were bred and maintained in a pathogen-free mouse colony at the Animal Department of the Gunma University School of Medicine. Five mice were injected i.v. into the tail vein with 370 kBq ^{18}F-MAT in 0.1 ml of saline. Brain and tumor tissue was taken at 5, 30, 60 and 120 min after injection with ^{18}F-AMT. Mice were killed and blood samples were taken. Brain and tumor tissue was dissected, weighed and counted for ^{18}F activity.

Fractionation of labeled acid-precipitable materials

To analyze metabolites, we employed the fractionation method described elsewhere.[2,14] The brain and tumor tissues were homogenized in ice-cold $HClO_4$ (0.4 M, 1 ml). The homogenized brain tissue was divided into the acid-soluble fraction (ASF) and acid-precipitable fraction (APF). The homogenate was centrifuged twice. The APF was processed for further determination of the uptake in lipid, RNA, DNA and protein. The flow chart of the fraction of ^{18}F-AMT is shown in Figure 1.

Lipid fraction

The precipitate was resuspended in 5 ml of $CHCl_3$:CH_3OH (2:1). After centrifugation at 300 r.p.m. for 10 min, the $CHCl_3$:CH_3OH phase was separated. The combined $CHCl_3$:CH_3OH fraction contained the radiolabeled lipids.

RNA fraction

The precipitate was dissolved in 1 ml of 0.3 N KOH. After incubation of the solution at 80 °C for 1 h to hydrolyze RNA, $HClO_4$ (3 N, 0.32 ml) was added and the mixture was kept on ice for 5 min. The precipitate

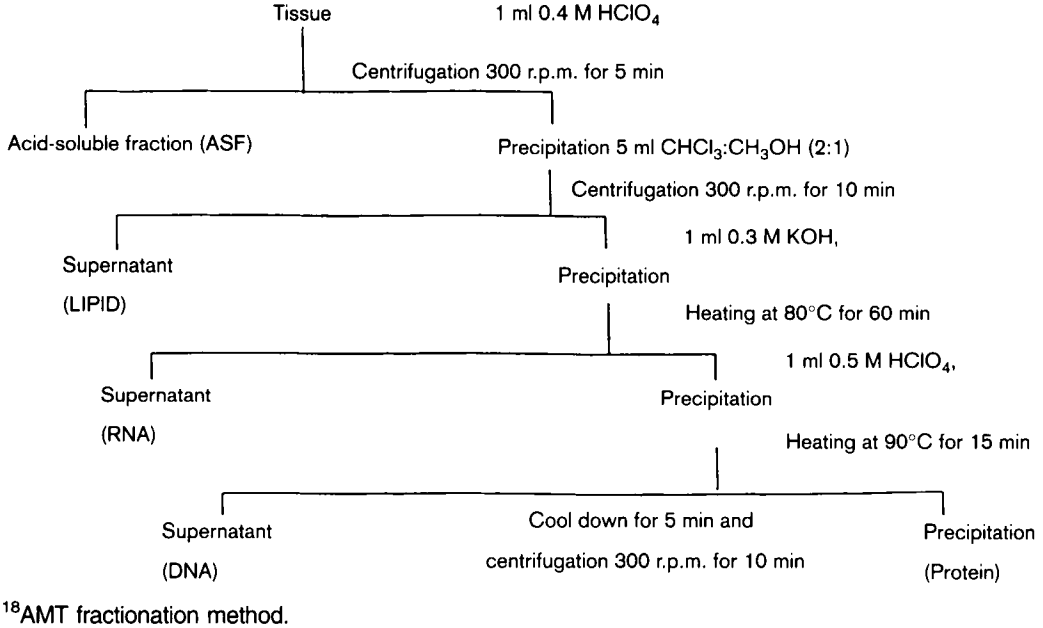

Figure 1. ^{18}FAMT fractionation method.

was then separated and washed with 1 ml of 0.5 N $HClO_4$ as described above. The combined supernatant was designated as the alkaline-labeled fraction containing the RNA hydrolyzate.

DNA and protein fractions

The precipitate was resuspended in 1 ml of 0.5 M $HClO_4$ and heated at 90°C for 15 min to hydrolyze the DNA. The solution was kept on ice for 5 min, and the precipitate was separated and washed with 0.4 M $HClO_4$ as described above. The combined supernatant and the final precipitate were labeled as the acid-labile fraction containing hydrolyzes of the DNA and protein fraction, respectively.

Analysis of metabolites in brain and tumors by thin-layer chromatography (TLC) and HPLC

TLC and HPLC were used to analyze metabolites in the brain and tumor tissue. The ASF was analyzed by HPLC and instant thin-layer chromatography (ITLC). A Lichrosorb RP18 (10 × 250 mm, GL Science, Tokyo, Japan) column with a mobile phase of 10% CH_3OH, 0.1% CH_3COOH at a flow rate of 6 ml/min was used to analyze the metabolites. Radioactivity and UV absorbance were measured by a Na-Tl scintillation 171 radioisotope detector and UV programmable module detector (Beckman, Allendale, NJ) at a wavelength of 280 nm, respectively. For ITLC, two mobile phases were used: saline and acetone. Saline development of ^{18}F-AMT gave a R_f from 0.9 to 1.0, whereas acetone gave a R_f from 0.4 to 0.5.

Autoradiography of rat brain

Sprague-Dawley rats (10 weeks old, female) were injected with 3.7 MBq (100 μCi) of ^{18}F-AMT through their tail vein and were killed at 60 min. Three groups were pretreated either with unlabeled AMT or vehicle prior to administering ^{18}F-AMT. In the first group, unlabeled AMT (200 mg/kg) was injected. The second group was injected with saline only and the third group served as a control group. After 2 h, brain was excised and frozen in dry ice powder immediately. The brain was sliced to 20 μm by a Cryostat microtome (Omihoshi, Tokyo, Japan). The Fuji film was exposed for 30 min and BAS 2000 (Fuji, Tokyo, Japan) was used to obtain images of the distribution of ^{18}F-AMT in brain.

Statistical analysis

Students *t*-test was used to analyze data. A two-tailed probability of $p < 0.05$ was considered to be statistically significant.

Results

Preparation of radiopharmaceuticals

Radiochemical purity of ^{18}F-AMT was more than 95% as determined by HPLC (shown in Figure 2). ^{18}F-AMT has two isomers, i.e. 3-^{18}F-AMT and 2-^{18}F-AMT, and the ratio was 6:1 by NMR analysis (data not shown). The final product containing both isomers was used for further studies without further purification.

In vivo distribution of ^{18}F-AMT in brain and tumor

In vivo distribution of the ^{18}F-AMT in acid the ASF and APF in tumor and brain at post-injection 5 and 60 min of ^{18}F-AMT is shown in Table 1. The uptake of ^{18}F-AMT in APF in brain was 21.9 ± 5.05 and 18.7 ± 4.75% both

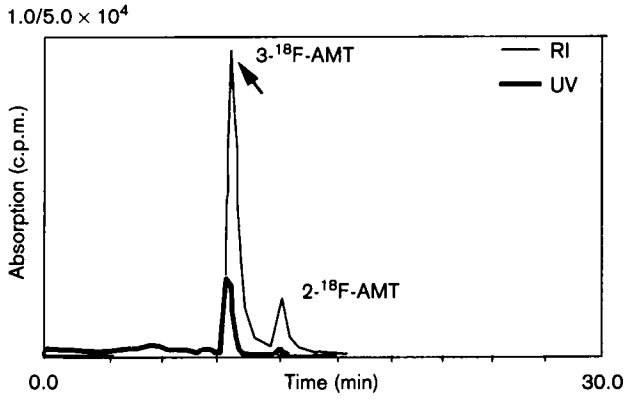

Figure 2. HPLC spectrum of ^{18}F-AMT.

Table 1. *In vivo* distribution of ^{18}F-AMT in the ASF and APF fraction at 5 and 60 min

Time (min)	ASF (%)	APF (%)
Brain fraction		
5 (*n*=4)	76.8 ± 5.5	21.9 ± 5.1
60 (*n*=4)	81.3 ± 4.8	18.7 ± 4.8
Tumor fraction		
5 (*n*=4)	88.1 ± 2.2	11.9 ± 2.3
60 (*n*=4)	88.8 ± 1.1	11.2 ± 1.1

at 5 and 60 min when compared to 11.8 ± 2.3 and $11.2 \pm 1.1\%$ in tumor at 5 and 60 min. There was more than 10% of APF uptake in the brain than that of tumor. These numbers implied that metabolism in brain tissue was more activated than in tumor.

Fractionation of the APF

Metabolic fractions of the ^{18}F-AMT between ASF and APF in brain tissue at 5 and 60 min are shown in Figure 3. APF was further divided into four fractions: lipid, RNA, DNA and proteins. Metabolic fractions of ^{18}F-AMT between ASF and APF in tumor tissue at 5 and 60 min are shown in Figure 3. The ASF in both brain and tumor tissues at 5 and 60 min was significantly higher than the APF ($p < 0.001$). Most of the radioactivity observed in the ASF was ^{18}F-AMT as determined by radio-TLC. The lipid fraction was the major component in both brain and tumor. The lipid fraction in brain was significantly different from the other three fractions at 60 min ($p < 0.01$). Similarly, the lipid

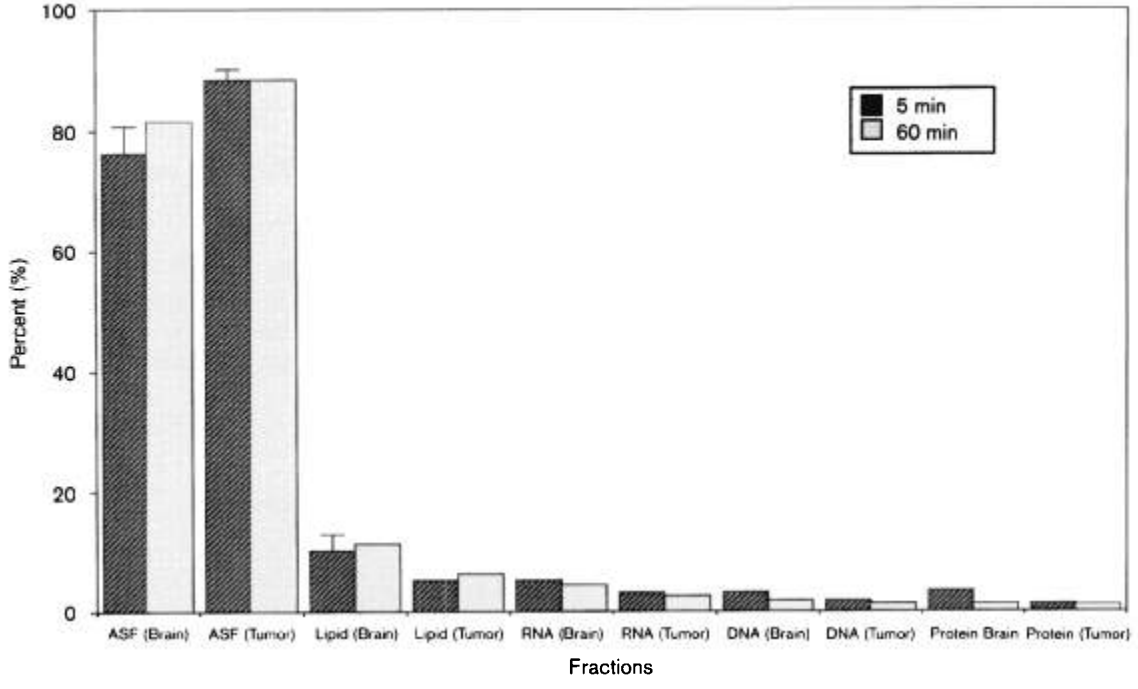

Figure 3. Mouse brain and tumor metabolite fractions at 5 and 60 min post-injection (n=4/each bar).

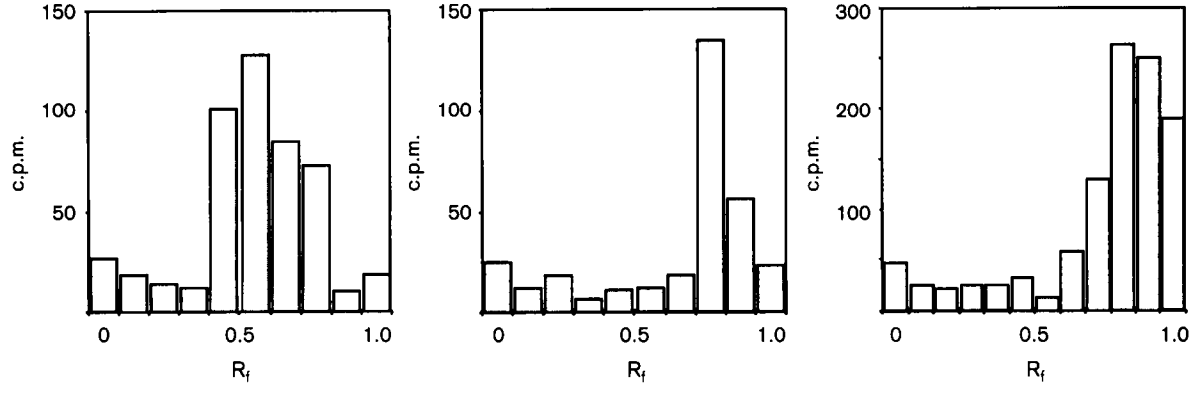

Figure 4. TLC of brain in acetone (left) and saline (center) solvent and tumor in saline solvent (right) at 30 min post-injection.

fraction in tumor was markedly different from the other three fractions both at 5 and 60 min ($p < 0.05$). The protein fraction of the uptake in both brain and tumor tissue was only 1-3% at 5 and 60 min post-injection. In particular, the lipid fraction uptake in brain, which was significantly different among the other three components, suggests that ^{18}F-AMT may be metabolized in a way similar to dopamine.[14,15]

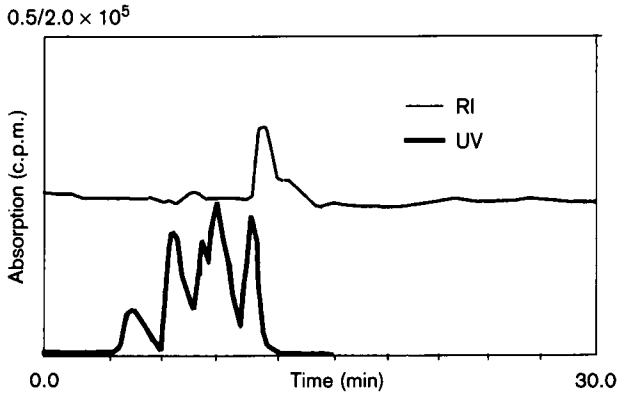

Figure 5. HPLC spectrum of brain metabolites at 30 min post-injection.

Analysis of metabolites in the ASF in brain and tumor

TLC using acetone and saline showed that there was one major peak in brain and tumor (shown in Figure 4). TLC in physiological saline solvents showed that there was one peak in brain and tumor. The peak detected in acetone at R_f 0.5-0.6 and in saline at R_f 0.9-1.0 is ^{18}F-AMT. Figure 5 showed that the solitary peak of ^{18}F-AMT could be detected by HPLC at 30 min post-injection in brain. There were several unknown peaks detected by UV at 280 nm; however, only ^{18}F-AMT could be detected by a radioactive detector.

Autoradiography of rat brain: competition of α-methyl-*p*-tyrosine (AMPT)

In Figure 6, a brain autoradiogram of a Sprague-Dawley rat (10 weeks female) showed that there was uptake of ^{18}F-AMT in rat brain at 60 min post-injection. ^{18}F-AMT had the highest uptake in the cortex and hippocampus (left and center). When rats were pretreated with AMPT and FAMT, there was a decreased uptake (right).

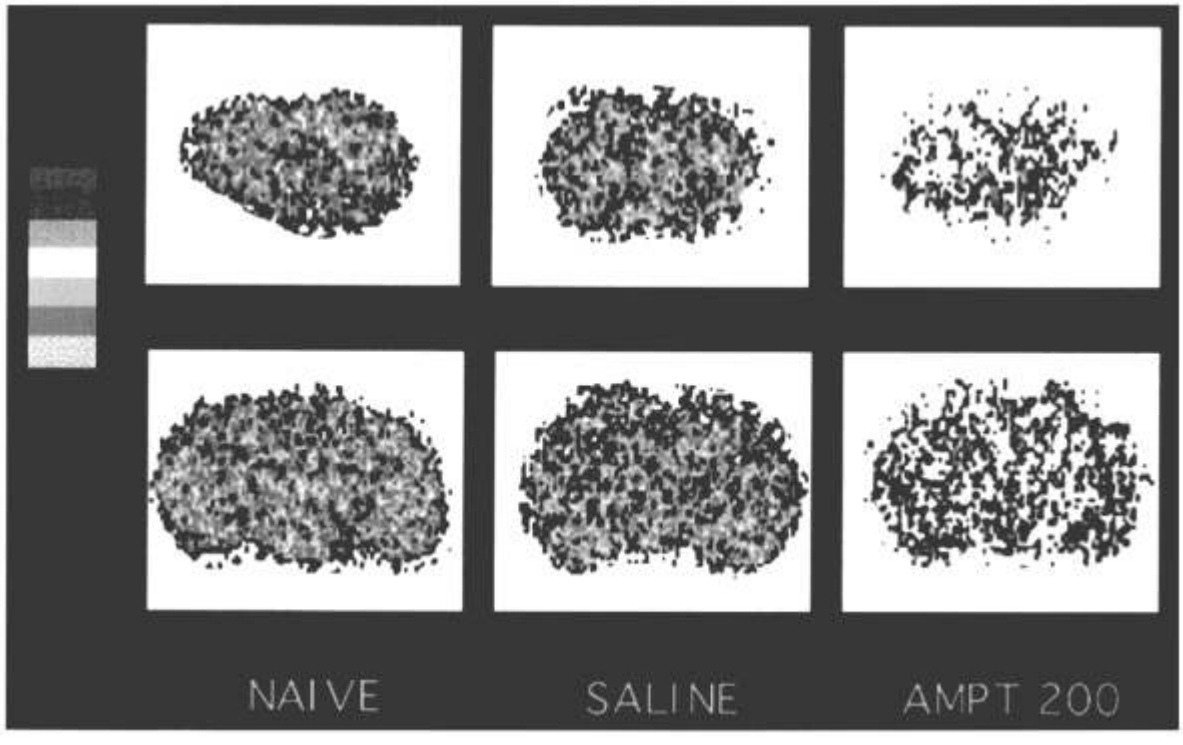

Figure 6. Autoradiography of ^{18}F-AMT on rat brain.

Discussion

Preparation of radiopharmaceutical

The presence of the methyl group in the α-position can enhance the stability of I-AMT against deiodination.[16] A newly synthesized ^{18}F-AMT amino acid radiopharmaceutical was shown to be stable in saline and human plasma up to 6 h as measured by HPLC.[11] Radiochemical purity of ^{18}F-AMT was more than 95%. Radiochemical yield of ^{18}F-AMT was improved to 40%. This gives enough radioactivity to examine patients for routine clinical studies.

In vivo distribution of ^{18}F-AMT in brain and tumor

In order to obtain good quality of images using PET, there are two influential factors, i.e. a high uptake of the tracer in the target tissue, and high count density ratios between the target and normal tissue. These two factors are influenced by blood flow and metabolism of the tracer in the target tissue. As for the uptake ratio of ^{18}F-AMT in tumor, ^{18}F-AMT was rapidly cleared from the kidneys.[13] The rapid clearance of ^{18}F-AMT from blood was accompanied by an increase in tumor uptake ratio. Our studies using LS180 tumor confirmed these findings and showed that maximal tumor uptake was sustained from approximately 30–60 min post-injection. The tumor uptake of ^{18}F-AMT at 60 min post-injection was significantly higher than that of FDG as reported in a previous paper.[12]

The biodistribution of ^{18}F-AMT in brain and tumor was 1.08 and 3.82% injected dose per gram tissue at 60 min, respectively.[13] However, the absolute percent injected dose per gram tissue uptake in tumor was more than 3 times the activity of that of brain.[13] The APF in brain was higher than that of tumor ($p<0.05$) at both 5 and 60 min. These differences could originate mainly from the metabolites in brain, which contains many enzymes such as tyrosine hydroxylase. ^{18}F-AMT is an inhibitor of the hydrolysis reaction and may be metabolized as catecholeamine metabolism. The metabolic fraction ratio of ^{18}F-AMT in the brain is higher than that in the tumor ($p<0.01$) both at 5 and 60 min. However, little changes in both brain and tumor between 5 and 60 min were observed ($p<0.01$). These results implied that ^{18}F-AMT was slowly metabolized and ^{18}F-AMT was taken up by the amino acid transport system in the cell during 60 min, followed by a slow diffusion back into the blood.

Fractionation of the APF

The tracer uptake of ^{18}F-AMT in tumor is significantly affected by tracer metabolism pathways, such as energy metabolism, protein synthesis and nucleic acid metabolism. ^{18}F-FDG has proven to be of value for cancer diagnosis based on the energy metabolism. Our ^{18}F-AMT is an amino acid analog, which is taken up by the amino acid transport system. Figure 3 represents metabolic fractions of the ^{18}F-AMT between ASF and four fractions, i.e. lipid, RNA, DNA and protein, in brain and tumor tissue at 5 and 60 min. As shown in Table 1, 88 and 81% of total activity in tumor and brain at 60 min, respectively, was not incorporated in any components. Statistically, little difference was seen between APF activity at 5 and 60 min in tumor but some metabolism had taken place in brain between 5 and 60 min ($p<0.01$). The lipid fraction was the major component in both brain and tumor involving metabolites. However, there was no significant difference among four tumor fractions at both 5 and 60 min. The lipid component in brain was statistically different from the three other components at 60 min ($p<0.01$). These data indicate that ^{18}F-AMT in the brain could be metabolized in a way similar to dopamine.

Analysis of metabolites in ASF in brain and tumor

Most of the amino acid is incorporated into protein such as [^{11}C]methionine and [2-^{18}F]tyrosine.[1,17] However, 80% of ^{18}F-AMT activity remained in the supernatant, which contains the metabolites from the enzyme in brain and tumor. We examined the metabolites in brain and tumor using HPLC and TLC. The incorporation of the tracer into the tissue proved to be less than 20% by the fractionation method. The fractionation method was applied to find the metabolism in tumor and brain into lipid, RNA, DNA and protein fractions. The metabolites, which remained in the supernatant, were also analyzed by HPLC and TLC. It turned out that tumor contains non-metabolites more than $88.1 \pm 2.2\%$, and that the remainder of about 12% was fractionated to lipid, RNA, DNA and protein. The first fraction contained water-soluble non-metabolite as determined by HPLC. Almost of all the activity was ^{18}F-AMT in the first fraction. Brain fractionation also was carried out. The first fraction contained $76.8 \pm 3.6\%$ total activity, and $10.2\% \pm 2.5\%$ was in lipid and 3–4% was in RNA, DNA and proteins, respectively.

HPLC and TLC showed almost all ^{18}F-AMT in the water-soluble fraction. These results suggested that ^{18}F-AMT was not metabolized in the tumor and brain but transported into the cell and only very slowly released from tumor and brain.

HPLC analysis showed a constant decrease of ^{18}F-AMT with time after 60 min; the quantification of transportation rate was possible using a two-compartment model. The proposed metabolic pathway of ^{18}F-AMT is an active transport system and 10% of ^{18}F-AMT in tumor is metabolized in four fractions, whereas 20% of ^{18}F-AMT in brain was mainly incorporated into lipid. There are many enzymes in the brain that catalyze catecholamine metabolism as well as protein synthesis. ^{18}F-AMT was taken up in cells and gradually diffused in blood at 30 and 60 min after injection. It was difficult to find metabolites by HPLC or TLC because of the low concentrations accumulated in the tissue after 30 and 60 min.

Autoradiography of rat brain: competition of AMT

The distribution of ^{18}F-AMT at 60 min is shown in an autoradiogram (Figure 1). The rat brain injected with ^{18}F-AMT had a high uptake in cortex and basal ganglia when pretreated by AMT; when then injected with ^{18}F-AMT, the uptake in rat brain decreased. Unlabeled AMT is a competitive inhibitor of ^{18}F-AMT. ^{18}F-AMT uptake was through a neutral amino acid transport system and was retained in the cells. It is well known that the transport of a specific amino acid into the brain can be inhibited by another amino acid that utilizes the same carrier system.[18] Our findings are consistent with the variable effect of amino acid loading reported by Ishiwata *et al.*[2]

Conclusion

A study of ^{18}F-AMT metabolism for LS180 tumor and brain in mouse was investigated. Fractionations of ^{18}F-AMT in brain and tumor in mice revealed that ^{18}F-AMT was hardly incorporated into proteins. These results are similar to that of ^{123}I-AMT as previously reported. HPLC and TLC results showed that there was almost only native ^{18}F-AMT in the brain and tumor. These results suggested that ^{18}F-AMT was not metabolized in the tumor and brain but transported into the cell followed by a very slow release. ^{18}F-AMT could be a promising tumor imaging agent for PET which enables quantification of amino acid transport.

Acknowledgments

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