



Biological evaluation of 3-[^{18}F]fluoro- α -methyl-D-tyrosine (D-[^{18}F]FAMT) as a novel amino acid tracer for positron emission tomography

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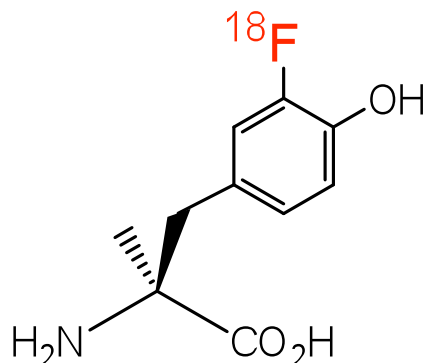
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1. Background and purpose

1-1 L-[^{18}F]FAMT and positron emission tomography (PET)

L-[^{18}F]FAMT (3-[^{18}F]fluoro- α -methyl-L-tyrosine)



- Amino acid tracer for PET imaging of tumors
- Higher specificity to tumor than [^{18}F]FDG
(Low accumulation in brain or inflammatory site)

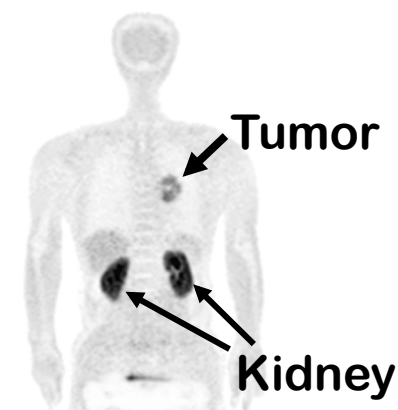
However...,

- Body clearance of L-[^{18}F]FAMT is slower than that of [^{18}F]FDG.
- L-[^{18}F]FAMT is highly accumulated and retained in the kidney.



Decrease of diagnostic accuracy

PET image
using L-[^{18}F]FAMT



1-2 Development of a novel PET tracer using D-amino acid

L-amino acids



D-amino acids

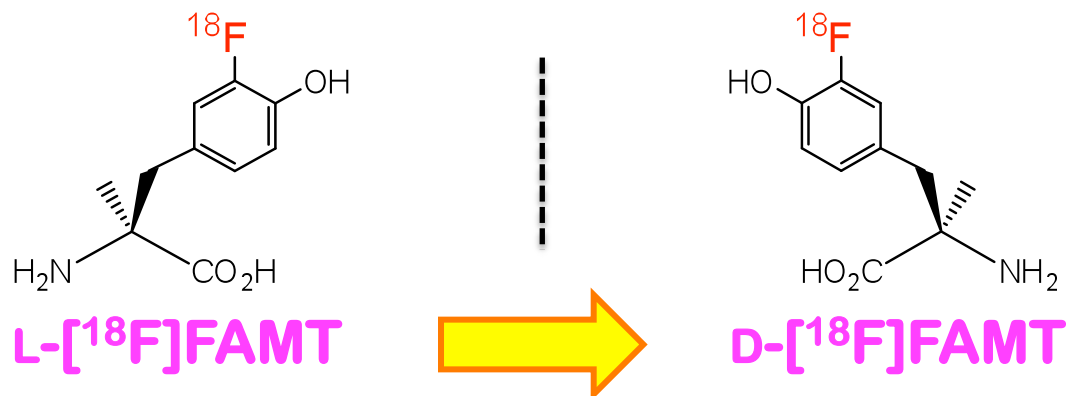
(unnatural amino acids)

● Previous reports have shown some favorable properties of D-amino acids for PET tracers.

Advantages of D-amino acids

- Rapid clearance from kidney to urine
- Low retention in non-target organs
- Accumulative in tumors

Thus, we expected that the D-isomer of FAMT (D-[^{18}F]FAMT) could facilitate body clearance and reduce renal accumulation of L-isomer.



In this study, D-[^{18}F]FAMT was synthesized and evaluated its usefulness.

2. Experiments and Results

2-1 Experimental design

To evaluate usefulness of D-[^{18}F]FAMT as a novel PET tracer, we carried out following experiments.

Experiments

1. Production of D- or L-[^{18}F]FAMT
2. *In vitro* and *in vivo* stability
3. Cellular uptake studies (Time-course)
4. Cellular uptake studies (mechanism of cellular uptake)
5. Biodistribution studies
6. Urinary excretion
7. PET imaging
8. Dosimetry

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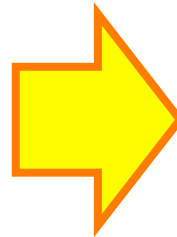
2.2 ^{18}F production and synthesis of $[\text{}^{18}\text{F}]\text{FAMT}$

Production of ^{18}F



(Biomedical Cyclotron CYPRIS HM-18)

Synthesis of D- or L- $[\text{}^{18}\text{F}]\text{FAMT}$



(FAMT automatic synthesizer)

Direct fluorination of α -methyltyrosine.
Reference: Nucl. Med. Commun.1997;18:169-175.

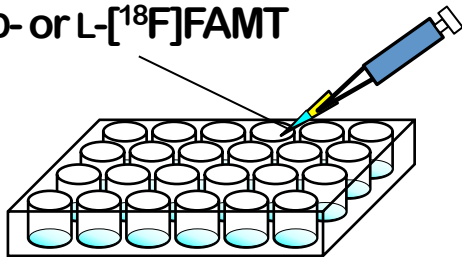
- Radiolabeling yield: **approximately 10%**
- Radiochemical purity: **96 ~ 99%**
- Specific activity: **120 GBq/mmol**
- No contamination with each enantiomer
- Stability: **High** (*in vitro* and *in vivo*)
(Over 95% of FAMT remained intact.)

Nuclear reaction	$^{20}\text{Ne} (\text{d}, \alpha) ^{18}\text{F}$
Target	^{20}Ne gas
Ion	Deuteron (10 MeV)
Irradiation	30 min

2.3 Cellular uptake of D- or L- [^{18}F]FAMT

Experimental method

D- or L- [^{18}F]FAMT

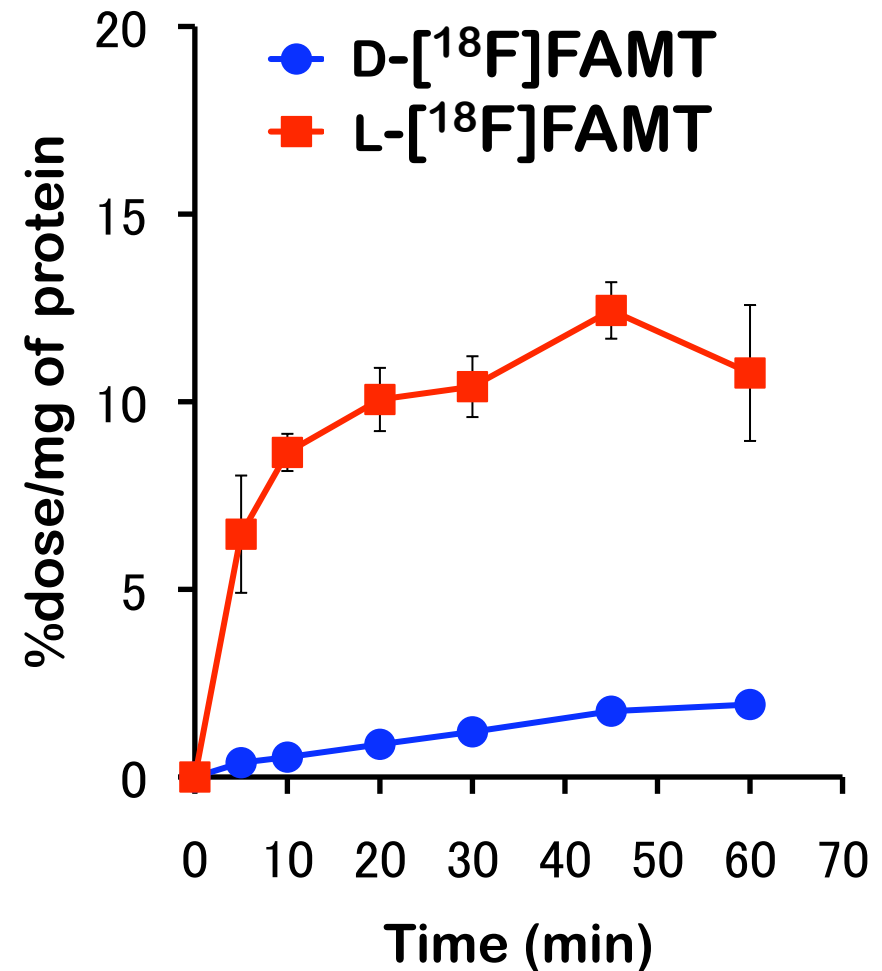


LS180 colon adenocarcinoma
 1.5×10^5 cells/well

Incubate for indicated time
Solubilized by 0.1 NaOH
Collect in sample tube

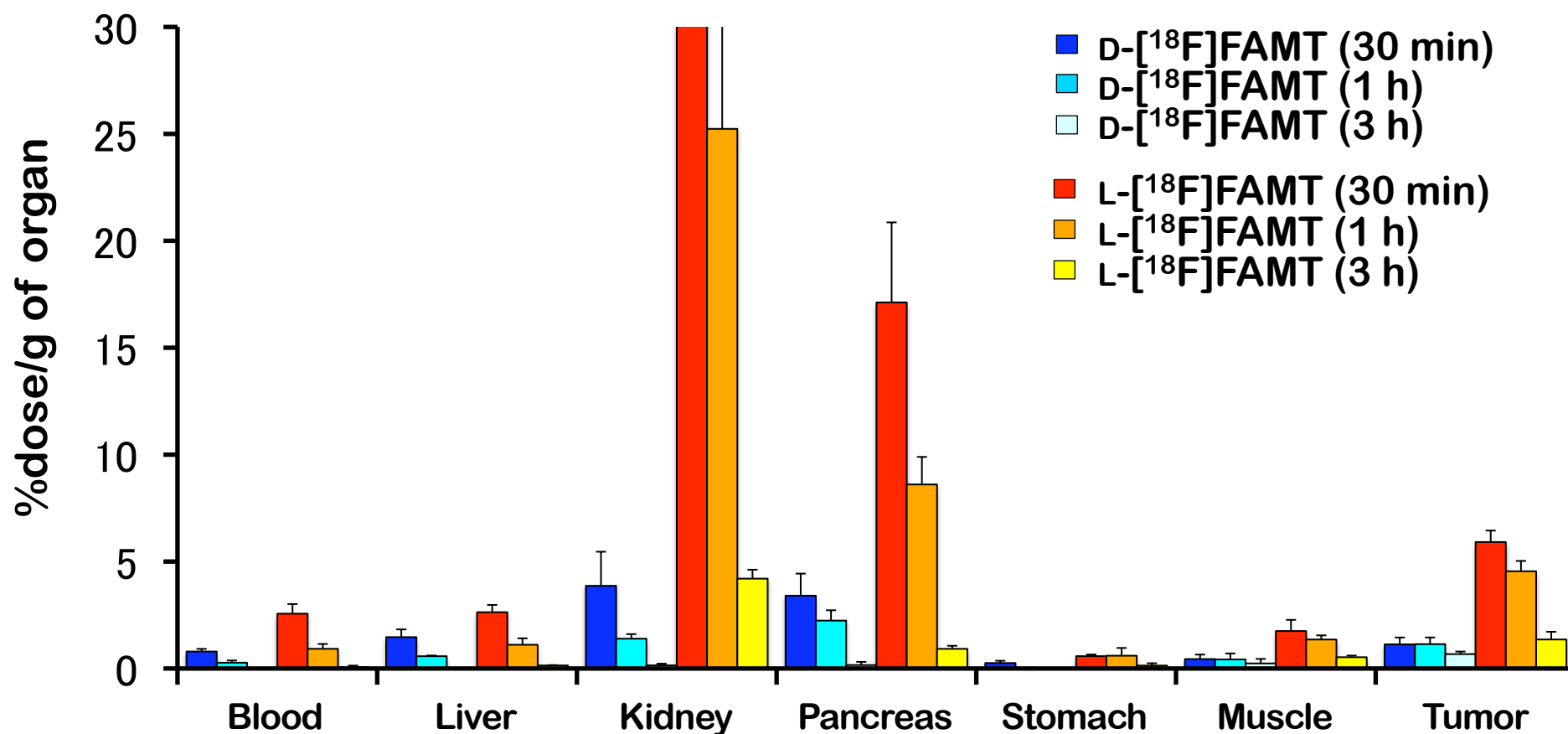


Measure radioactivity



Cellular uptake
D- [^{18}F]FAMT \ll L- [^{18}F]FAMT

2.4 Biodistribution in tumor-bearing mice



D-[¹⁸F]FAMT showed

- ◆ Rapid clearance from the blood
- ◆ Low distribution to normal organ (especially kidney)
- ◆ Low distribution to tumor

2.5 Tumor-to-blood (T/B) and tumor-to-muscle (T/M) ratios

To expect the contrast of tumor to background in PET image, T/B and T/M ratios were calculated from radioactivity in the organs.

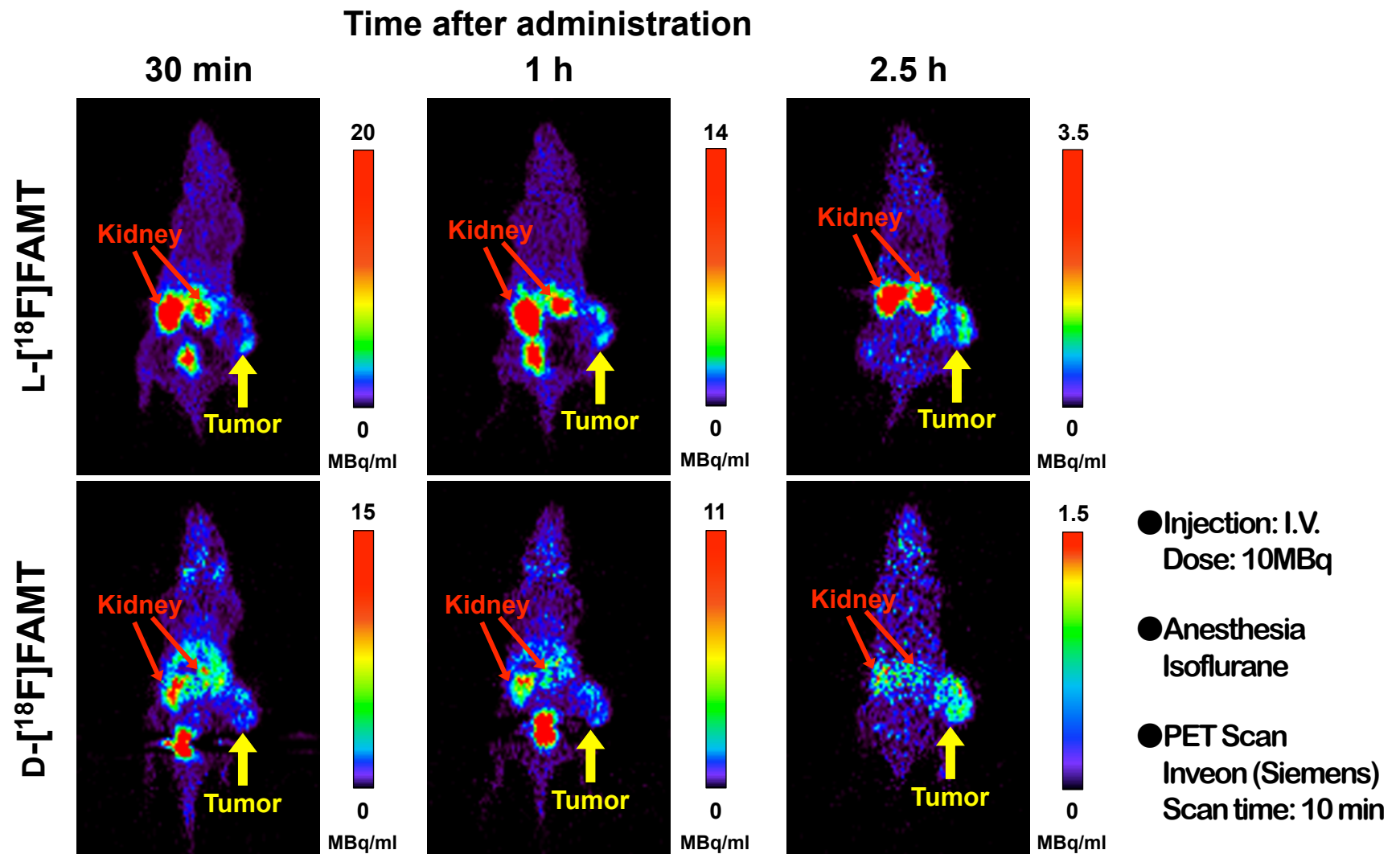
	30 min	1 h	3 h
T/B ratio			
D-[¹⁸ F]FAMT	1.45 ± 0.47	4.35 ± 0.82	* Not calculated
L-[¹⁸ F]FAMT	2.37 ± 0.51	5.13 ± 1.17	13.13 ± 3.92
T/M ratio			
D-[¹⁸ F]FAMT	3.19 ± 1.96	3.72 ± 2.36	2.12 ± 0.46
L-[¹⁸ F]FAMT	3.61 ± 1.06	3.37 ± 0.25	2.54 ± 0.36

* Because the radioactivity of D-[¹⁸F]FAMT was decreased to the background level.

T/B ratio and T/M ratio
D-[¹⁸F]FAMT \doteq L-[¹⁸F]FAMT

The contrast of D-[¹⁸F]FAMT would be similar to that of L-[¹⁸F]FAMT in PET imaging.

2.6 PET imaging using D- or L- [^{18}F]FAMT



Accumulation and retention in the kidney: $\text{D-}[^{18}\text{F}]\text{FAMT} \ll \text{L-}[^{18}\text{F}]\text{FAMT}$
PET using $\text{D-}[^{18}\text{F}]\text{FAMT}$ enabled clear visualization of the tumors.

3. Conclusions

Summary of D-[^{18}F]FAMT in this study

- (1) D-[^{18}F]FAMT was successfully synthesized.
- (2) D-[^{18}F]FAMT was **highly stable**.
- (3) Cellular uptake of D-[^{18}F]FAMT was low and slow.
- (4) D-[^{18}F]FAMT was **rapidly cleared** from the body.
- (5) D-[^{18}F]FAMT was **rarely distributed and retained** in normal organs.
- (6) Tumor accumulation of D-[^{18}F]FAMT was low,
but T/B and T/M ratios were similar to those of L-[^{18}F]FAMT.
- (7) PET using D-[^{18}F]FAMT provided **a clear visualization of tumor**.



D-[^{18}F]FAMT could potentially serve as a novel PET tracer for imaging of malignant tumors.

4. Perspectives

The future studies of D- ^{18}F FAMT are shown as follows.

- Differences of the mechanism in renal accumulation between D- and L- ^{18}F FAMT.
- PET imaging of renal or urological tumor using D- ^{18}F FAMT.

5. Acknowledgements

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I'd like to express my great appreciation to my co-workers.

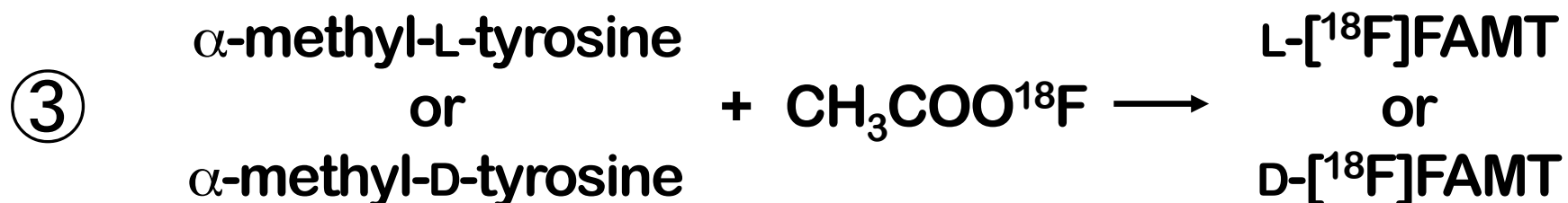


Thank you for your kind attention !!



Supplemental slide 1 Fluorination of α -methyltyrosine

Fluorination step of α -methyltyrosine

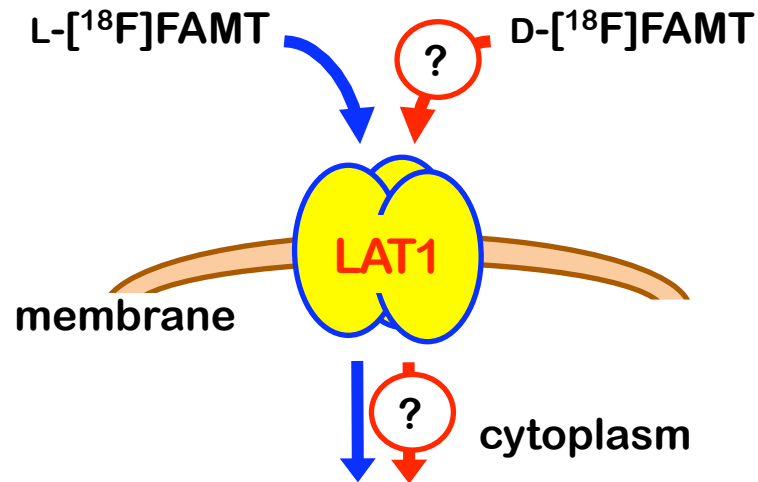


Ref., Tomiyoshi K, *et al.* Nucl Med Commun. 1997;18:169-75.

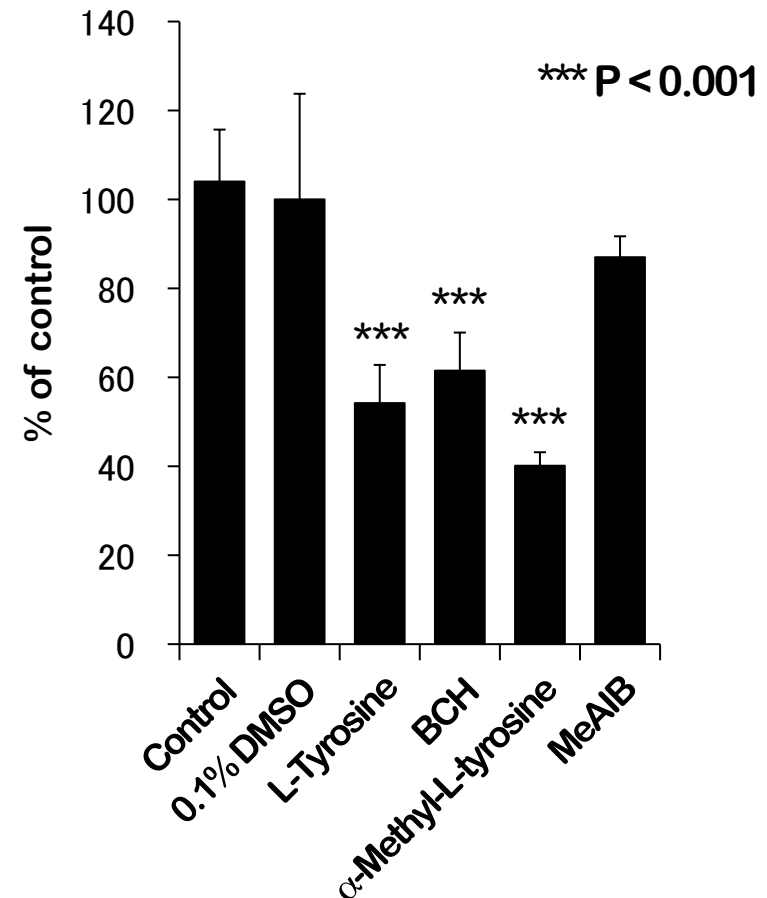
Synthesis of isomers of ^{18}F -labelled amino acid radiopharmaceutical: position 2- and 3- ^{18}F - α -methyltyrosine using a separation and purification system.

Supplemental slide 2 Mechanism of cellular uptake

Cellular uptake of L-[¹⁸F]FAMT



LAT1:
System L amino acid transporter 1
(High expression in various cancer)



- α-Methyl-L-tyrosine: selective inhibitor of LAT1
- BCH: selective inhibitor of system L
- MeAIB: selective inhibitor of system ASC

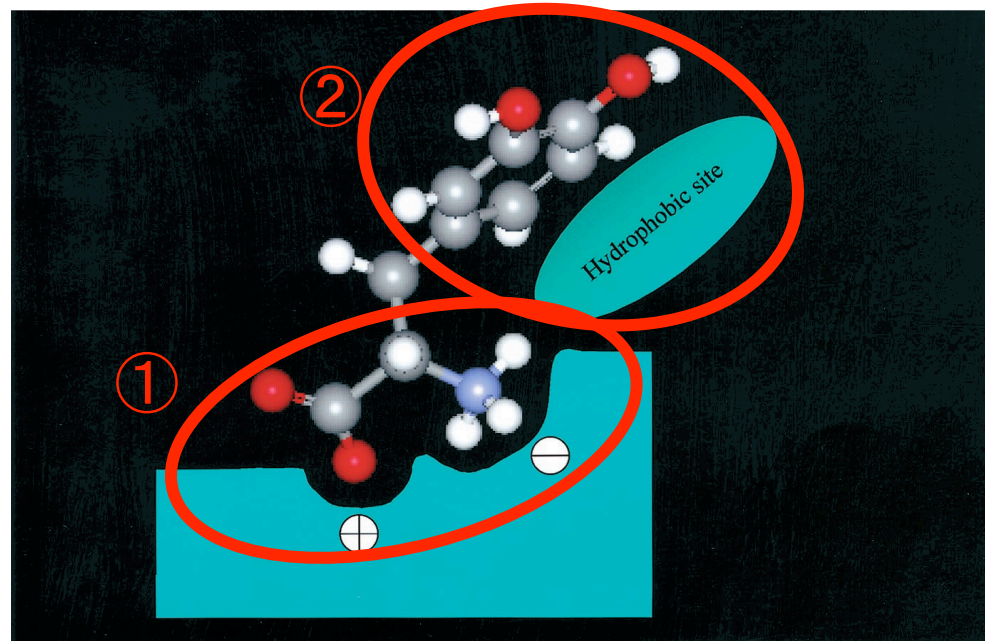
LAT1 is involved in cellular uptake of D-[¹⁸F]FAMT.

The expression of LAT1 is also confirmed by RT-PCR and immunoblotting.

Supplemental slide 3 Mechanism of cellular uptake

Proposed model for the substrate-binding site of LAT1.

Reference: Uchino H. et al. Mol. Pharmacol. 61:729–737, 2002



There are two interactions involved in L-amino acid recognition by LAT1.

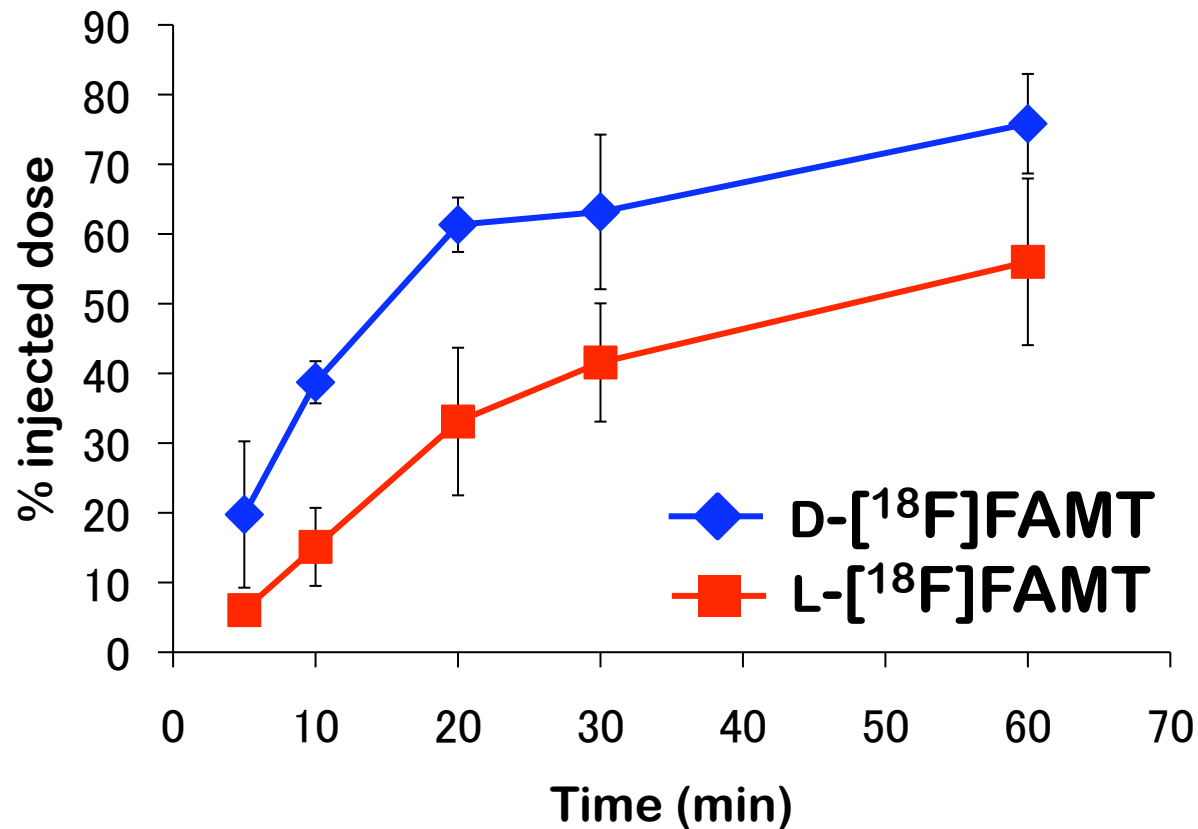
- ① Electronic interaction ② Hydrophobic interaction



Both interactions would be difficult with the D-isomers
because of the conformational differences

Supplemental slide 4 Urinary excretion

To confirm rapid excretion of D-[^{18}F]FAMT, radioactivity in the urine was measured.



Urinary excretion

D-[^{18}F]FAMT >> L-[^{18}F]FAMT

Supplemental slide 5 Dosimetry

Since D-[^{18}F]FAMT was rapidly cleared from the body, we estimated exposure dose of D- or L-[^{18}F]FAMT using calculation code.

Calculation code

Organ Level Internal Dose Assessment (OLINDA)

Nuclide: F-18

Model: Adult Male

Kinetics: Biodistribution data, such as time, % injected dose, and target organ masses were substituted for kinetic model and fit them to a function.



L-[^{18}F]FAMT: Effective Dose: 2.73×10^{-3} mSv/MBq

D-[^{18}F]FAMT: Effective Dose: 7.74×10^{-4} mSv/MBq

D-[^{18}F]FAMT decreases exposure dose to approximately $\frac{1}{4}$ of L-[^{18}F]FAMT.