Tumor Uptake Studies of S-Adenosyl-L-[methyl-11C]Methionine and L-[methyl-11C]Methionine

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Introduction

In recent in vivo tumor studies with positron emission tomography (PET), much attention has been paid to amino acid metabolism and protein synthesis. Especially, a significance of L-[methyl-^{11}C]methionine ([^{11}C]Met) has been demonstrated in the studies of brain and lung tumors.[1-7] Bustany and coworkers presented a kinetic model for the quantitative measurement of protein synthesis rates.[6] However, the metabolism of [^{11}C]Met is rather complex,[8-11] although the main metabolic pathway is protein incorporation. One of minor pathways is the conversion of methionine to S-adenosyl-L-methionine (SAM). Only methionine is an in vivo precursor of SAM, which plays an important role in biochemical transmethylation processes. On the other hand, the SAM is a propylamine donor of polyamine biosynthesis, which is enhanced in connection with increased DNA synthesis in growing tumor tissues. In the tumor tissues a high concentration of the SAM is also reported.

In this paper we present the tumor uptake of [^{11}C]SAM in mice and rats and compare metabolic fates of the [^{11}C]SAM and [^{11}C]Met in tumor and other tissues.

Materials and Methods

Radiopharmaceuticals

L-[methyl-^{11}C]Methionine ([^{11}C]Met) and S-adenosyl-L-[methyl-^{11}C]-methionine ([^{11}C]SAM) were synthesized as described previously.[12]

Animal studies

C3H/He mice bearing transplantable mammary carcinoma (FM3A) were injected with [^{11}C]SAM (200 μCi/10 nmol) via a lateral tail vein. At various times after injection the mice were sacrificed, and their organs were dissected. The ^{11}C radioactivity was measured using a gamma-counter. The uptake was expressed as the differential absorption radio (DAR), (counts/g tissue) × (g body weight/total injected counts). In Donryu rats bearing transplantable ascitic hepatoma (AH109A) [^{11}C]SAM (400 μCi/80 nmol) were injected

intravenously Another group of AH109A-bearing rats were also injected with [^{11}C]Met (2 mCi/60 nmol). Tissue uptakes of two tracers were measured as described above.

In case of mice injected with [^{11}C]SAM, FM3A tumor, liver or kidney tissue was divided to acid-soluble and acid-precipitable fractions in order to estimate the amount of [^{11}C] SAM involved in the transmethylation into macromolecules

In case of rats injected with [^{11}C]SAM or [^{11}C]Met, for parts of AH109A, liver, kidney and brain tissue the same procedure was used to separate the acid-soluble and acid-precipitable fractions. Other parts of tissues were used for measuring the amount of [^{11}C]SAM involved in the transmethylation into lipids.[12)]

Results and Discussion

The results of tissue distribution studies of [^{11}C]SAM in tumor-bearing mice and rats are presented in Tables 1 and 2. After administration of [^{11}C]SAM, slightly different distribution patterns were found for mice and rats. For both animals the blood clearance of ^{11}C was rapid and the highest uptake was observed in the kidney. In mice, the levels of ^{11}C in the FM3A tissue was relatively high and remained nearly constant. In other organs the levels of ^{11}C decreased with time. In rats, AH109A showed a high uptake and the levels of ^{11}C in this tissue, pancreas, spleen and small intestine increased with time. Probably, these slight differences depend on the metabolism of SAM in rats and mice. For both animals tumor-to-organ ratios increased with time in several organs including the liver, and high ratios were obtained at 60 min after injection, especially in the brain, muscle and blood. These results indicate that because of high requirement of SAM in tumor tissue the ^{11}C-labeled SAM has potential as a positron-emitting tracer for tumor localization with PET.

In Table 3 the incorporation rates of ^{11}C radioactivity into the acid-precipitable fraction are summarized in FM3A tumor, liver and kidney tissue. The proportion of the acid-precipitable fraction increased with time for all three tissues. In the liver tissue more than 50 % of ^{11}C was detected in this fraction at 30 min after injection. Also about a third of ^{11}C was observed in this fraction of tumor and kidney tissue. These results indicate that the ^{11}C-methyl group of SAM is not only transferred into many biologically active small molecules but also into macromolecules, i. e. nucleic acids and protein.

To compare the metabolism of [^{11}C]SAM and [^{11}C]Met, the tissue distributions and the proportions of ^{11}C radioactivity in the acid-precipitable and in the lipid fractions were measured in AH109A-bearing rats at 20 min after injection. Blood level of the ^{11}C after injection of [^{11}C]Met was much lower compared to [^{11}C]SAM. In AH109A tissue the ^{11}C level of [^{11}C]SAM was about two third of that of [^{11}C]Met. In the AH109A tumor, liver and kidney tissue the ratios of ^{11}C in the acid-precipitable fraction after

injection of [^{11}C]SAM were less than those for [^{11}C]Met. In case of [^{11}C]SAM the 28.6 % of ^{11}C was detected in the lipid fraction of liver. The corresponding figures were 7.9 % and 5.5 % in the AH109A and kidney, respectively. On the other hand, for the [^{11}C]Met the ratio of the lipid fraction in liver was much larger compared to the [^{11}C]SAM, but in kidney, AH109A tumor and brain tissue the ratios of this fraction were very low. These results suggest that the high uptake of [^{11}C]SAM in the tumor tissue is explained by the enhanced transmethylation processes, in which the methylation of macromolecules is significantly important.

From these results several characteristics were also presented in the metabolism of [^{11}C]Met. In the liver most ^{11}C radioactivity of [^{11}C]Met is incorporated into the acid-precipitable fraction but a main part of this fraction present the lipids in membrane and high lipophilic macromolecules. In the tumor tissue the amount of ^{11}C in the lipid fraction is very low. The acid-precipitable fraction presents mainly the newly synthesized protein. However, it is reasonable to consider that a certain amount of ^{11}C is expected to be incorporated into the acid-precipitable fraction via [^{11}C]SAM. After injection of L-[methyl-^{14}C]methionine the presence of ^{14}C-labeled SAM was confirmed in plasma, tumor and brain,[8-11] and this radioactive methyl group is rapidly incorporated into the acid-precipitable fraction in the tumor tissue (Table 3, 4). Many reports demonstrate the significance of Met not only in protein synthesis but also in methylation processes in tumor cells. Although in the present studies metabolism of [^{11}C]SAM in the brain tissue was not investigated because of difficult transport of [^{11}C]SAM through the blood-brain barrier, significance of Met as a precusor of SAM is clear.[8] These findings suggest that for in vivo measurement of protein synthesis by combination of [^{11}C]Met and PET the minor metabolic pathways such as the conversion of [^{11}C]Met to [^{11}C]SAM should be taken count for a kinetic model.

In conclusion this paper present that the [^{11}C]SAM has potential as a radiopharmaceutical for tumor localization with PET and that the uptake of this tracer may reflect the enhanced transmethylation processes in the tumors.

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Table 1. Tissue distribution of ^{11}C after intravenous injection of S-adenosyl-L-[methyl-^{11}C]methionine in tumor-bearing mice.

	Uptake, DAR Tumor-to-organ ratio				
	1 min (n=3)	5 min (n=3)	10 min (n=3)	30 min (n=3)	60 min (n=3)
Blood	3.02 ± 0.04	1.96 ± 0.04	1.02 ± 0.17	0.22 ± 0.02	0.11 ± 0.01
	0.23	0.34	0.40	2.17	5.55
FM3A	0.68 ± 0.14	0.67 ± 0.12	0.40 ± 0.09	0.47 ± 0.16	0.63 ± 0.06
	1.00	1.00	1.00	1.00	1.00
Brain	0.18 ± 0.08	0.11 ± 0.033	0.055± 0.007	0.034± 0.001	0.036± 0.012
	3.74	8.56	7.28	13.7	17.7
Heart	1.34 ± 0.09	0.73 ± 0.05	0.43 ± 0.00	0.23 ± 0.03	0.11 ± 0.01
	0.51	0.91	0.94	2.02	5.56
Lung	2.53 ± 1.62	1.89 ± 0.15	0.87 ± 0.14	0.34 ± 0.01	0.25 ± 0.03
	0.27	0.35	0.46	1.37	2.49
Liver	0.64 ± 0.04	0.46 ± 0.01	0.41 ± 0.06	0.25 ± 0.01	0.27 ± 0.13
	1.06	1.45	0.99	1.86	2.38
Pancreas	1.07 ± 0.20	0.82 ± 0.12	0.69 ± 0.15	0.36 ± 0.04	0.16 ± 0.07
	0.64	0.81	0.59	1.30	3.89
Spleen	0.78 ± 0.08	0.73 ± 0.06	0.64 ± 0.23	0.21 ± 0.04	0.10 ± 0.02
	0.78	0.92	0.63	2.21	6.23
Small intestine	1.10 ± 0.15 0.62	0.80 ± 0.15 0.84	0.66 ± 0.16 0.61	0.49 ± 0.03 0.96	0.62 ± 0.24 1.02
Kidney	6.84 ± 0.92	11.14 ± 1.44	7.82 ± 1.23	8.20 ± 1.31	5.25 ± 0.42
	0.10	0.06	0.05	0.06	0.12
Muscle	0.52 ± 0.10	0.48 ± 0.08	0.26 ± 0.03	0.11 ± 0.02	0.051± 0.012
	3.74	8.56	7.28	13.7	17.7

Table 2. Tissue distribution of ^{11}C after intravenous injection of S-adenosyl-L-[methyl-^{11}C]methyionine in tumor-bearing rats.

	Uptake, DAR Tumor-to-organ ratio		
	5 min (n=3)	20 min (n=3)	60 min (n=3)
Blood	1.63 ±0.03	1.39 ±0.06	0.50 ±0.06
	0.52	0.84	4.10
AH109A	0.85 ±0.04	1.18 ±0.07	2.05 ±0.20
	1.00	1.00	1.00
Brain	0.14 ±0.02	0.11 ±0.01	0.17 ±0.03
	6.10	11.1	11.8
Heart	0.77 ±0.02	0.72 ±0.06	0.51 ±0.05
	1.11	1.63	4.01
Lung	1.57 ±0.27	1.32 ±0.18	1.06 ±0.09
	0.54	0.89	1.94
Liver	0.77 ±0.03	1.63 ±0.12	1.33 ±0.06
	1.10	0.72	1.55
Pancreas	1.11 ±0.17	1.69 ±0.02	1.85 ±0.20
	0.77	0.70	1.11
Spleen	0.66 ±0.07	0.77 ±0.04	0.89 ±0.05
	1.29	1.53	2.30
Small intestine	0.74 ±0.03	1.96 ±0.70	3.79 ±0.47
	1.14	0.60	0.54
Kidney	18.84 ±1.92	23.90 ±2.91	18.73 ±2.78
	0.05	0.05	0.11
Muscle	0.53 ±0.01	0.43 ±0.03	0.47 ±0.10
	1.62	2.75	4.38

Table 3. Percemtage of ^{11}C radioactivity in the acid-precipitable fraction in mice tissue after intravenous injection of S-adenosyl-L-[methyl-^{11}C]-methionine.

	5 min (n=3)	10 min (n=3)	30 min (n=3)	60 min (n=3)
FM3A	14.6 ± 0.9	17.7 ± 4.7	30.8 ± 4.1	38.2 ± 8.7
Liver	26.4 ±10.9	32.3 ± 6.1	58.2 ± 9.1	55.0 ±12.2
Kidney	17.9 ± 1.5	19.6 ± 0.2	28.7 ± 1.3	40.6 ± 0.9

Table 4. The tissue level of ^{11}C radioactivity and the percentage of ^{11}C radioactivity in the acid-precipitable (APF) and chloroform-methanol fractions (CMF) in rat tissues at 20 min after intravenous injection of S-adenosyl-L-[methyl-^{11}C]methionine or L-[methyl-^{11}C]methionine.

	S-Adenosyl-L-[methyl-^{11}C]methionine			L-[methyl-^{11}C] Methionine		
	Uptake (DAR) n=3	APF (%) n=3	CMF (%) n=2	Uptake (DAR) n=5	APF (%) n=3	CMF (%) n=3
Blood	1.39 ± 0.06			0.27 ± 0.04		
AH109A	1.18 ± 0.07	26.8 ± 3.5	7.9	1.79 ± 0.32	61.3 ± 0.7	2.2 ± 1.0
Liver	1.63 ± 0.12	54.4 ± 9.3	28.6	4.21 ± 1.24	91.0 ± 0.7	66.5 ± 2.6
Kidney	23.90 ± 2.91	36.2 ± 1.5	5.5	2.16 ± 0.30	61.2 ± 16.6	4.2 ± 0.4
Brain	0.11 ± 0.01			0.47 ± 0.01	73.3 ± 17.3	1.2 ± 1.0