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Relationship between [^{14}C]MeAIB uptake and amino acid transporter family gene expression levels or proliferative activity in a pilot study in human carcinoma cells: Comparison with [^{3}H]methionine uptake

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ABSTRACT

Introduction: To clarify the difference between system A and L amino acid transport imaging in PET clinical imaging, we focused on the use of α-[*N*-methyl-**^{11}C**]-methylaminoisobutyric acid ([**^{11}C**]MeAIB), and compared it with [*S*-methyl-**^{11}C**]-L-methionine ([**^{11}C**]MET). The aim of this study was to assess the correlation of accumulation of these two radioactive amino acid analogs with expression of amino acid transporters and cell proliferative activity in carcinoma cells.

Methods: Amino acid uptake inhibitor studies were performed in four human carcinoma cells (epidermal carcinoma A431, colorectal carcinoma LS180, and lung carcinomas PC14/GL and H441/GL) using the radioisotope analogs [^{3}H]MET and [^{14}C]MeAIB. MeAIB was used to inhibit the A system and 2-amino-2-norbornane-carboxylic acid (BCH) was used to inhibit the L system. The carcinoma gene expression levels of a number of amino acid transporters were measured by microarray and quantitative polymerase chain reaction. Carcinoma proliferative activity was assessed using accumulation of [methyl-^{3}H]-3'-deoxy-3'-fluorothymidine ([^{3}H]FLT).

Results and conclusion: [^{14}C]MeAIB uptake occurred principally via a Na^{+}-dependent A type mechanism whereas [^{3}H]MET uptake occurred predominantly via a Na^{+}-independent L type mechanism although other transporters were also utilized depending on cell type. There was no correlation between [^{3}H]MET uptake and total system L amino acid transporter (LAT) expression. In contrast, [^{14}C]MeAIB uptake strongly correlated with total system A amino acid transporter (SNAT) expression and proliferative activity in this preliminary study using four human carcinoma cell lines. Carcinoma proliferative activity also correlated with total SNAT expression.

Advances in Knowledge and Implications for Patient Care: Because there is a significant correlation between the accumulation of [^{14}C]MeAIB and the gene expression level of total SNAT as well as the accumulation of [^{3}H]FLT, it is suggested that use of the analog [^{11}C]MeAIB in PET may provide an indication of tumor cell proliferative activity. [^{11}C]MeAIB is therefore expected to be very useful in PET imaging.

1. Introduction

2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG), an analog of glucose, is the most commonly used radiopharmaceutical in positron emission tomography (PET)-CT imaging [1]. PET-CT imaging is based on the preferential uptake of [^{18}F]FDG in tumor cells as compared to normal cells, because glucose metabolism is increased in tumor cells. [^{18}F]FDG PET-CT has been found to be useful in lesion detection and characterization, evaluation of tumor stage, assessment of treatment response and detection of recurrent disease [2,3]. However, the specificity of this technique is low in patients with active infections and inflammatory diseases (because of high FDG uptake in macrophages) and in the brain (because of high background FDG uptake) [4,5]. Therefore, the development of post-FDG radiopharmaceuticals is needed.

After glucose transport, amino acid transport is another important pathway in cellular energy metabolism. Therefore, natural or artificial amino acid analogs have been widely studied clinically as potential

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post-FDG radiopharmaceuticals for PET imaging; one of the most important radiolabeled amino acids in this regard is [*S*-methyl-^{11}C]-L-methionine ([^{11}C]MET) [6,7]. Since both amino acid transport and protein synthesis rates are enhanced in tumors, [^{11}C]MET has been as widely used in brain tumor imaging as *O*-(2-[^{18}F]-fluoroethyl)-L-tyrosine ([^{18}F]FET) [8,9].

Numerous amino acid transporters have been identified at the molecular level and have been characterized in mammalian cells [10–12]. The main transport systems for the uptake of neutral amino acids are the A, L, and alanine-serine-cysteine (ASC) amino acid transport systems. System L amino acid transporters are Na^+ independent, and are the main transport mechanism for methionine, tyrosine, phenylalanine, and their analogs such as FAMT (3-fluoro-α-methyl-tyrosine) [13–16]. The system A and ASC amino acid transporters are Na^+-dependent, however, compared to the system L transporters, their involvement in the transport by radiolabeled amino acids in nuclear medicine has not been studied in detail.

The artificial amino acid radiopharmaceutical α-[*N*-methyl-^{11}C]-methylaminoisobutyric acid ([^{11}C]MeAIB) is a promising specific substrate of system A amino acid transport. Compared with [^{11}C]MET, [^{11}C]MeAIB is metabolically stable [17] and it has been studied both pre-clinically and clinically. For example, [^{11}C]MeAIB has been shown to be useful in the measurement of amino acid uptake into skeletal muscle and in the diagnosis of malignant lymphoma and head and neck cancers [18–20]. In our institute, [^{11}C]MeAIB PET has proven useful in the diagnosis of chest diseases, especially in the differential diagnosis between sarcoidosis and metastasis [21].

Previous studies in carcinoma cells have shown that there is a high correlation between both MET and FAMT uptake and the gene expression levels of system L amino acid transporters [15,16,22]. However, the relationship between the accumulation of radiolabeled amino acids and the gene expression levels of system A amino acid transporters has not been examined.

In this study, we explored the amino acid transport systems in four different human carcinoma cell lines using α-[1-^{14}C]-methylaminoisobutyric acid ([^{14}C]MeAIB) as a substrate and comparing it to [*S*-methyl-^{3}H]-L-methionine ([^{3}H]MET) and [methyl-^{3}H]-3'-deoxy-3'-fluorothymidine ([^{3}H]FLT). We elected to use ^{14}C or ^{3}H–labeled amino acid analogs because both [^{11}C]MeAIB and [^{11}C]MET have a very short half-life (20 min). We also investigated the gene expression profiles of numerous amino acid transporters in these four types of human carcinoma cell lines using microarray analysis. Following on from this initial screen we used quantitative reverse transcription polymerase chain reaction (qRT-PCR) to characterize the expression of the mRNAs encoding SNAT1, SNAT2, SNAT4, ASCT1, ASCT2, y^+LAT1, y^+LAT2, LAT1, LAT2, LAT3, LAT4, and 4F2hc. We then examined the correlation between accumulation of [^{3}H]MET and the quantitative mRNA expression of total system L transporter (total LAT = LAT1 + LAT2 + LAT3 + LAT4) and total system A amino acid transporters (total SNAT = SNAT1 + SNAT2 + SNAT4) in these human carcinoma cell lines. Finally, we examined the correlation between accumulation of [^{3}H]FLT uptake and [^{14}C]MeAIB uptake as well as the correlation between [^{3}H]FLT uptake and the quantitative gene expression of total SNAT transporters.

2. Materials and methods

2.1. Radiolabeled amino acid analogs and amino acid transport inhibitors

Because of the short half-life of ^{11}C (20 min), radiolabeled amino acid analogs with much longer half-lives were used instead. ^{14}C–labeled MeAIB ([^{14}C]MeAIB, 37 kBq/ml), ^{3}H–labeled MET ([^{3}H]MET, 18.5 kBq/ml) and ^{3}H–labeled FLT ([^{3}H]FLT, 18.5 kBq/ml) were obtained from American Radiolabeled Chemicals Inc. (St Louis, Missouri, USA). MeAIB (α-methylaminoisobutyric acid), a specific inhibitor of system A, and BCH (2-amino-2-norbornane-carboxylic acid), a specific inhibitor of system L, were acquired from Sigma-Aldrich Japan KK (Tokyo, Japan).

2.2. Cell culture

Cell line studies were performed using a modification of the methods described by Shikano et al. and Nakajima et al. [23,24], as follows. A431 and LS180 cell lines were purchased from Dainippon Sumitomo Pharma Co., Ltd. (Osaka, Japan). The cultured human tumor cell lines H441 and PC14 were obtained from the University of Texas, MD Anderson Cancer Center, Houston, TX, USA. All cells were cultured in 150 mm cell culture dishes (Becton Dickinson, New Jersey, USA)in a 5% CO_2 humidified atmosphere at 37 °C with specific media as follows; A431 cells were maintained in Dulbecco's modified Eagle"'s medium (DMEM; Sigma-Aldrich, Japan) containing high glucose supplemented with 10% fetal bovine serum (FBS) and 3.7 g/L $NaHCO_3$, H441 cells were maintained in RPMI-1640 medium (Sigma-Aldrich, Japan) supplemented with 10% FBS, 1% sodium pyruvate (Sigma-Aldrich, Japan) and 2.0 g/L $NaHCO_3$, PC14 cells were maintained in DMEM/Nutrient Mixture F-12 Ham (DMEM/F12; Sigma-Aldrich, Japan) supplemented with 10% FBS and 1.2 g/L $NaHCO_3$, and LS180 cells were maintained in minimum essential medium Eagle (MEM; Sigma-Aldrich, Japan) supplemented with 10% FBS, 1% sodium pyruvate and 2.2 g/L $NaHCO_3$. Sub-culturing was performed every five days using 0.02% EDTA and 0.05% trypsin. For amino acid uptake experiments, cells were seeded into a 24-well Multiwell Plate (Becton Dickinson, New Jersey, USA) at a density of 5×10^5 cells/well and were used 24 h after plating.

2.3. Measurement of [^{14}C]MeAIB, [^{3}H]MET and [^{3}H]FLT transport in human carcinoma cells

For transport studies in a sodium-containing medium, phosphate-buffered saline (PBS) pH 7.4 (137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1.5 mM K_2HPO_4, 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$) was used. For sodium-free transport studies, the NaCl and Na_2HPO_4 in PBS were replaced with the same concentrations of choline-Cl and K_2HPO_4, respectively. After removal of the culture medium, the 24-well Multiwell Plate was washed once with 5 mL of incubation medium for 10 min at 37 °C. The cells were then incubated with 500 μL/well of incubation medium containing [^{14}C]MeAIB (37 kBq/mL) and [^{3}H]MET (18.5 kBq/mL) or [^{3}H]FLT (18.5 kBq/mL) for 10 min at 37 °C. For the experiment involving amino acid transport, inhibitors were added to a final concentration of 1 mM, and the cells were then incubated for 10 min at 37 °C with [^{14}C]MeAIB (37 kBq/mL) and [^{3}H]MET (18.5 kBq/mL) or [^{3}H]FLT (18.5 kBq/mL). After incubation with the radiolabeled amino acid analog, the medium was aspirated and the monolayers were rapidly rinsed twice with 500 μL of ice-cold incubation medium. Cells were solubilized in 500 μL of 0.1 N NaOH, and the radioactivity (either ^{3}H and ^{14}C) of an aliquot (400 μL) was measured by addition of Clear-Sol II (Nacalai Tesque Inc., Kyoto, Japan) and scintillation counting using an LSC-5100 liquid scintillation counter (Hitachi Aloka Medical, Ltd., Tokyo, Japan).

To characterize relative contributions of each type of transport system to overall amino acid analog uptake, we performed inhibition experiments with inhibitors as described above. BCH was used as a system L inhibitor and MeAIB was used as a system A inhibitor. To calculate the relative contributions of amino acid transporter systems, we used the methods reported by Shikano et al. [23] and Nakajima et al. [24]. In brief, uptake of [^{3}H]MET and [^{14}C]MeAIB in the absence of inhibitors was used as the control (100%). System A uptake was calculated as [control uptake in Na^+-PBS] – [uptake in the presence of MeAIB in Na^+-PBS]. Uptake by system ASC and/or other systems (for example IMINO, B^0, G-like, N, and y^+L which cannot be individually assessed yet) was calculated as [uptake in the presence of MeAIB in Na^+-PBS] – [control uptake in Na^+ free-PBS]. System L uptake was calculated as [control uptake in Na^+ free-PBS] – [uptake in the presence of BCH in Na^+ free-PBS].