Intratumoral distribution of ^{64}Cu-ATSM and ^{18}F-FDG in VX2 tumor bearing rabbit model.

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1. Introduction

Imaging acquisition and analysis of hypoxic region within solid tumor is essential for understanding the microenvironment of tumor, and is also important for the establishment of proper therapeutic strategy and evaluation for radiation therapy (1-5). ^{64}Cu-labeled diacetyl-bis (N_4-methylthiosemicarbazone) (^{64}Cu-ATSM) is a promising agent for imaging of hypoxic tissues and internal radiation therapy for tumor. In this study, we obtained PET/CT images of tumor using ^{64}Cu-ATSM and ^{18}F-FDG, and then evaluated the distribution of hypoxic region after comparing with oxygen partial pressure in VX2 tumor bearing rabbit model. MR images are also obtained for precise anatomical information.

2. Methods and Results

2.1 Animal Preparation

Total 4 rabbits of 3 kg body weight (New Zealand white rabbit, female) were used in this study. VX2 tumors were produced by implantation of finely chopped tumor tissues from already established tumor bearing rabbits (provided by Seoul National University Hospital) to thigh muscle. Tumor bearing rabbit was anesthetized by intramuscular injection of a mixture of Zoletil (150 μl/kg) and Domitor (50 μl/kg) (3:1).

2.2 Image acquisition

PET and MR images were obtained in 2 or 3 weeks after tumor implantation. ^{64}Cu-ATSM PET images were obtained at 1 day after ^{18}F-FDG PET image acquisition in every week. ^{18}F-FDG of 1 mCi and ^{64}Cu-ATSM of 2.5 mCi were administered intravenously.

2.2.1 PET/CT

PET/CT images were obtained using clinical PET/CT scanner (Biograph True Point TrueV, Siemens). CT image was acquired using the following parameters; X-ray tube voltage: 130 kVp, current: 40 mA, exposure time: 600 ms, 512 × 512 matrix, pixel size: 1.36 mm and slice thicknesses: 5 mm). PET scan was performed using iterative reconstruction ordered-subset expectation maximization (OSEM) algorithm (iteration: 4, subset: 16) with CT based attenuation correction.

2.2.2 MRI

MR images were obtained with a 3T clinical scanner (MAGNETOM Tim Trio, Siemens). T1-weighted imaging using a T1-weighted VIBE sequence the following conditions (TR: 11.60 ms, TE: 3.37 ms, FA: 10°, FOV: 100 x 90.6 mm, image matrix: 290 x 320, voxel size: 0.3 x 0.3 x 1.0 mm, average: 1, slice: 40) were taken by coronal view. T2-weighted turbo spin echo sequence images using the following conditions: (TR: 3150 ms, TE: 38 ms, FA: 120 °, FOV: 100 x 100 mm, image matrix: 320 x 320, voxel size: 0.3 x 0.3 x 1.5 mm, average: 2, slice: 16) were taken by coronal view. After injection of Gd-based contrast agent, Omniscan (Gadodiamide, Gd-DTPA-BMA), the contrast enhanced T1-weighted images were obtained.

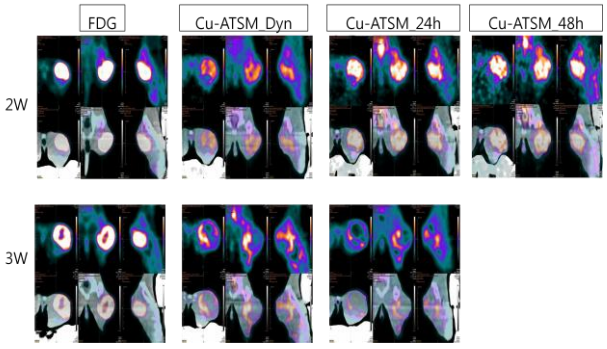


Fig 1. PET/CT and MR co-resistration images were obtained in 2 or 3 weeks after transplantation.

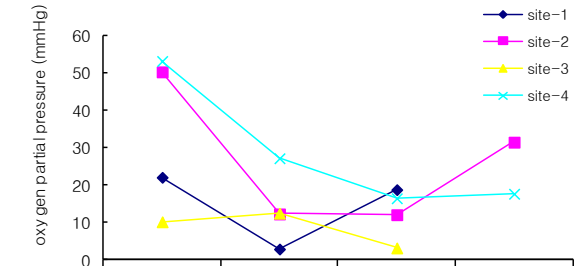
In VX2 tumors, the intratumoral distribution of ^{64}Cu–ATSM displayed a significant temporal evolution up to 48 h after injection. In this study, the distribution of ^{64}Cu-ATSM was corresponded to that of ^{18}F-FDG only at late time (24 – 48 h) images.

2.3 Image analysis

Acquired PET images were reconstructed for the calculation of maximum standardized uptake value (SUVmax) at region of interest (ROI) using Inveon Research Workplace (IRW). The uptake of ^{18}F-FDG was not correlated (r^2= 0.27, $p<0.001$) with early ^{64}Cu-ATSM uptake, but showed very significant correlation (r^2= 0.95, $p<0.001$) with ^{18}F-FDG at 48 h after injection.

2.4 Oxygen probe measurements

Oxygen partial pressure in tumor tissue was measured by using OXYLITE O2 probe at depth of 4 steps with 10 mm interval on randomly selected 4 sites.



	1cm	2cm	3cm	4cm
site-1	22.1	2.92	18.8	
site-2	50.3	12.3	12.1	31.5
site-3	10.2	12.5	3.21	
site-4	53.2	27.2	16.5	17.7

Fig 2. Partial pressure of oxygen measured toward the central region of the tumor was found to be hypoxic.

Oxygen probe measurements were broadly consistent with ^{18}F-FDG and late ^{64}Cu-ATSM images.

2.5 Autoradiography

At 1 h before sacrifice, 1 mCi of ^{18}F-FDG was administered intravenously, and the extracted-tumor tissue was frozen at -80℃. The frozen section of tumor was performed on 5 mm thickness by Auto Cryotome, and exposure images were obtained by BAS image plate.

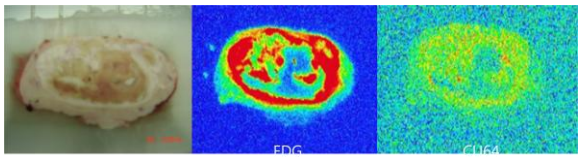


Fig 3. Photograph and autoradiographic images. The activity of radiotracer (^{18}F-FDG and ^{64}Cu-ATSM) in the tumor slice was detected using a BAS scanner.

The major accumulation of ^{64}Cu-ATSM was observed around the outer rim of the tumor masses of which consisted mainly of active cells and expected to be hypoxic. ^{18}F-FDG was distributed more widely with highest levels in the inner regions where pre-necrotic cells were mainly observed.

3. Conclusions

In our study, it was revealed that two regions with different characteristics within one tumor mass could be distinguished by ^{64}Cu-ATSM and ^{18}F-FDG. ^{64}Cu-ATSM could provide more specific information about the tumor regions where viable cells exist than ^{18}F-FDG. ^{64}Cu-ATSM can also delineate the hypoxic fraction and can be a useful tool to detect “hypoxic but viable” regions in tumors, one of the primary targets in the treatment of tumors.

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