Correlation of Glut-1 and Glut-3 expression with F-18 FDG uptake in pulmonary inflammatory lesions

Zhen Guang Wang, MD*, Ming Ming Yu, Ph.Da, Yu Han, MSb, Feng Yu Wu, MSa, Guang Jie Yang, Ph.Da, Da Cheng Li, MSa, Si Min Liu, MSa

Abstract
The aim of the study was to investigate the correlation of glucose transporter-1 (Glut-1) and glucose transporter-3 (Glut-3) expression with F-18 FDG uptake in pulmonary inflammatory lesions. Twenty-two patients with pulmonary inflammatory lesions underwent positron emission tomography/computed tomography (PET/CT) examination preoperatively, and Glut-1 and Glut-3 expression were detected by immunohistochemistry in these lesions. Correlations of Glut-1 and Glut-3 with 18F-FDG uptake were assessed using Spearman’s rank correlation test. The maximum standardized uptake value (SUVmax) of pulmonary inflammatory lesions in 22 patients was 0.50 to 7.50, with a mean value of 3.66 ± 1.62. Immunohistochemical staining scores of Glut-1 and Glut-3 were 2.18 ± 0.96 and 2.82 ± 1.37, respectively. The expression of Glut-1 and Glut-3 was positively correlated with F-18 FDG uptake. Glut-3 expression was evidently higher than Glut-1 expression in 22 patients.

Glut-1 and Glut-3 expressions are high in pulmonary inflammatory lesions, and Glut-3 plays a more important role in F-18 FDG uptake in pulmonary inflammatory lesions.

Keywords: F-18 fluorodeoxyglucose, glucose transporter-1, glucose transporter-3, pulmonary inflammatory lesion

1. Introduction
F-18 fluorodeoxyglucose (F-18 FDG) positron emission tomography/computed tomography (PET/CT) is mainly used for tumor diagnosis and staging. Inflammatory cells with high glycometabolism, such as lymphocytes, granulocytes, and macrophages, also show high F-18 FDG uptake, resulting in false positive F-18 FDG PET/CT imaging and reduced effectiveness of PET/CT for differential diagnosis between benign and malignant lesions. Thus, it is of great value to investigate the mechanism of F-18 FDG uptake in inflammatory lesions.

Glucose transporters (Glut) are the main carriers for glucose transport and are widely distributed in various human tissues and cells. Glucose transporter-1 (Glut-1) and glucose transporter-3 (Glut-3) present high affinity to glucose and are closely related with glycometabolism.[13–14] With this regard, it is necessary to understand the relationship between F-18 FDG PET/CT imaging characteristics of pulmonary inflammatory lesions and Glut-1, 3 expression in these lesions.

2. Methods
2.1. Patients
The study was approved by the Ethical Review Boards of the Affiliated Hospital of Qingdao University. Informed consent was obtained from all patients included in the study. Twenty-two patients (male = 13, female = 9, age = 37–81 years old, median age = 60) with pulmonary inflammatory lesions confirmed by pathology after surgery from November 2010 to December 2014 were enrolled in this study, including 10 cases of chronic pulmonary inflammation, 5 cases of pulmonary tuberculosis, 4 cases of fungal granulomatous lesions, 2 cases of inflammatory pseudotumor, and 1 case of focal organizing pneumonia.

2.2. F-18 FDG PET/CT scan
F-18 FDG PET/CT was performed on these 22 patients. The patient fasted for at least 6 hours before the intravenous injection of F-18 FDG at a dose of 0.2mCi/kg. After resting for 60 minutes, the patients were given 300 to 500 mL of pure drinking water followed by bladder emptying before the PET/CT examination (GE Discovery VCT, GE Healthcare, Milwaukee, WI). Before positron emission tomography (PET) scanning, full-body computed tomography (CT) scanning from the skull base to the mid-femurs was acquired for attenuation correction and anatomic localization. The
PET scanning conditions were as follows: during quiet breathing, the scan time for each bed position was 3 minutes. For the diagnostic chest CT scan, the patient was trained in breath holding before the examination, and the scan was conducted from the thoracic inlet to the adrenal level with inspiratory breath hold.[5]

2.3. Detection of Glut-1 and Glut-3 expressions

Immunohistochemical staining was performed to determine the expression of Glut-1 and Glut-3. A positive result was determined as yellow granules that appeared on the cell membrane or in the cytoplasm. Five fields of view were selected randomly on each slice under high magnification (×400), and 200 cells were quantified in each field. The percentage of positive cells on each slice was calculated. The score was 0 when the positive percentage was < 1%, the score was 1 when the positive percentage was 1% to 10%, the score was 2 when the positive percentage was 11% to 50%, the score was 3 when the positive percentage was 51% to 80%, and the score was 4 when the positive percentage was > 80%. The following scores were used for color intensity, 0 = colorless, 1 = light yellow, 2 = dark yellow, and 3 = brown. The total staining score was the sum of positive rate score and color intensity score: 0 to 1 (−), 2 to 3 (+), 4 to 5 (++), and 6 to 7 (+++). These results were considered positive when the total staining score was ≥ 2.

2.4. Statistical analysis

The t-test was used to compare the means of the measurement data, and Spearman’s rank correlation test was used to analyze the correlation among different indicators. A statistically significant difference was established when p < 0.05. The statistical software used in this study was SPSS17.0.

3. Results

3.1. Correlation between the maximum diameter and the maximum standardized uptake value

Of the 22 cases of pulmonary inflammatory lesions, the maximum diameter (Dmax) was 0.81 to 5.30 cm, and the mean value was 2.63 ± 1.36 cm. The maximum standardized uptake value (SUVmax) was 0.50 to 7.50, and the mean value was 3.66 ± 1.62. The result of the statistical analysis indicated no correlation between Dmax and SUVmax (P = 0.43).

3.2. Expression of Glut-1 and Glut-3

Expression of Glut-1: Results of the Glut-1 staining in pulmonary inflammatory lesions in the 22 cases showed that the positive rate was 82% (18/22), and the total staining scores were 0 to 3, with a median of 2, including 4 cases with a final score of 0 to 1, and 18 cases with a final score of 2 to 3.

Expression of Glut-3: Results of the Glut-3 staining in pulmonary inflammatory lesions in the 22 cases showed that the positive rate was 91% (20/22), and the total staining scores were 0 to 5, with a median of 3, including 2 cases with a final score of 0 to 1, 14 cases with a final score of 2 to 3, and 6 cases with a final score of 4 to 5.

3.3. Correlation between F-18 FDG uptake and the expression of Glut-1 and Glut-3

There was a positive correlation between Glut-1 expression and the SUVmax of all 22 patients with pulmonary inflammatory lesions (r = 0.639, P = 0.001), and the Glut-3 expression (r =

Figure 1. A 51-year-old man presented with a pulmonary nodule in the right lower lobe of the lung. Diagnosis of pulmonary cryptococcosis was confirmed by pathological evidence. (A) Thin-section CT shows a pulmonary nodule in the right lower lobe of the lung with lobulation, spiculation, and pleural indentation. (B) 18F-FDG PET imaging shows high radioactivity uptake in the nodule in the right lower lobe of the lung with an SUVmax of 4.8. (C) Glut-1 expression in the nodule (×200 magnification, weakly positive). (D) Glut-3 expression in the nodule (×200 magnification, strongly positive). CT = computed tomography, 18F-FDG = fluorine 18-fluorodeoxyglucose, Glut-1 = glucose transporter type 1, Glut-3 = glucose transporter type 3, PET = positron emission tomography, SUVmax = maximal standardized uptake value.
0.773, \( P < 0.001 \). Glut-3 expression was significantly higher compared to Glut-1 expression (\( P < 0.001 \)) (Fig. 1).

4. Discussion

Fludeoxyglucose (FDG) is a glucose analog that can be transported into cytoplasm by Glut and subsequently phosphorylated by hexokinase (HK) to FDG-6-phosphate, which cannot continue to metabolize or be transported out of the cells. Thus, F-18 FDG PET imaging can be performed based on intracellular stagnation. Currently, there are 13 members of the Glut family, including 5 glucose transporter proteins on the cell membrane. Under normal physiological conditions, Glut-1 and Glut-3 have a relatively high affinity with glucose and play an important role in cellular metabolism.

Some studies have proposed that the mechanism underlying high uptake of F-18 FDG in pulmonary inflammatory lesions is increased demands of energy due to the activation of inflammatory cells by inflammation stimuli.[6–11] Thus, the concentration of F-18 FDG in the inflammation lesion is related to inflammation activity. Davis et al.[12] found that active pulmonary tuberculosis mainly presented high uptake of F-18 FDG, whereas treated or obsolete pulmonary tuberculosis mainly presented nonuptake or mild/intermediate uptake of F-18 FDG (SUV < 2.5). These results are consistent with the findings of this study in which a few pulmonary inflammatory lesions were found to exhibit obvious uptake of F-18 FDG in PET imaging, for example, 5 cases of untreated tuberculosis (TB) in this study contained a large number of inflammatory cells, including lymphocytes, monocytes, and macrophages in the pathological slices of the lesions. The majority of pulmonary inflammatory lesions presented relatively lower F-18 FDG uptake in PET imaging, with blurred edges or even a “halo sign.” In the pathological slices, the cells appeared very sparse, with infiltration of a large number of inflammatory cells. These findings further support the uptake of F-18 FDG by inflammatory cells visualized using PET imaging.[10–11]

How do inflammatory cells increase the uptake of F-18 FDG? In this study, stained slices revealed the expression of Glut-1 and Glut-3 mainly on the cell membrane or in the cytoplasm of inflammatory cells, including lymphocytes, monocytes, and macrophages. The expression of Glut-1 and Glut-3 in these pathological tissues was detected, and these results indicated low F-18 FDG uptake and low expression of Glut-1 and Glut-3 in most of the inflammatory lesions. However, a relatively higher F-18 FDG uptake and expression of Glut-1 and Glut-3 were observed in some of the inflammatory lesions, in which the expression level of Glut-3 was much higher compared to Glut-1, suggesting that Glut-3 and Glut-1 both participated in the uptake of F-18 FDG in inflammatory lesions while Glut-3 played a more important role in F-18 FDG uptake. Some studies have found that Glut can be expressed in the cell nucleus, cytoplasm, and cell membrane, and can be transferred from the cytoplasm to the cell membrane when the cellular glucose demand is higher, to improve the efficiency of glucose transportation.[7,12] It can be speculated that inflammatory reactions in pulmonary inflammatory lesions cause the infiltration of inflammatory cells, increased expression of Glut-1 and Glut-3 in inflammatory cells, an improved efficiency of glucose transportation for more F-18 FDG uptake, and PET imaging of a part of the pulmonary inflammatory lesions. However, the mechanism of the upgraded expression of Glut in pulmonary inflammatory lesions is still unclear. Fu et al.[13] found equivalent expression of Glut-1 and Glut-3 in lymphocytes by mRNA analysis of Glut receptors in inflammatory cells, but following the activation of inflammatory factors, Glut-1 increased to 3.5 times, whereas Glut-3 increased to 6 times. Moreover, the expression of Glut-1 in monocytes was 88% of the level in lymphocytes, whereas Glut-3 was 8.4 times compared to levels observed in lymphocytes. However, after the activation of inflammatory factors, Glut-1 increased to 1.9 times, whereas Glut-3 decreased to 32% to 45% of the original level. Macrophages are transformed from monocytes, and following this transformation, Glut-1 decreased to 60% of the original level, whereas Glut-3 increased to 3.5 times of the original amount together with a specific amount of Glut-5. Thus, the expressions of Glut-3 and Glut-1 in the inflammatory lesions are related to the type, quantity, and degree of activation of the inflammatory cells.

Nomori et al.[14] performed a comparison of the false positive lesions with different sizes in the lungs and found that the main problem of the lesion < 1 cm was false negative, whereas the lesion with a diameter of 1 to 3 cm presented more false positive results, of which the false positive rates of the PET image was as high as 35%. Thus, it was concluded that the size of the lesions could be one of the influential factors of F-18 FDG uptake. In the 22 cases of pulmonary inflammatory lesions in this study, the SUV_{max} values were between 0.50 and 7.50, and the cases of mild F-18 FDG uptake (SUV_{max} < 2.5) were 27.3% (6/22), and 72.7% (16/22) cases presented intermediate or high F-18 FDG uptake. Of all the 22 lesions, 14 lesions were ≤3 cm, whereas 8 lesions were larger than 3 cm, with the uptake of F-18 FDG of the latter higher compared to the former. However, the differences in the SUV_{max} values were not statistically significant. This finding might be due to the down-regulated Glut receptors on the cell membrane of the inflammatory cells, the smaller amount of inflammatory cells and the fibroblasts as a main cell component in larger lesions. Thus, the type, quantity, and the degree of activation of the inflammatory cells in the pulmonary inflammatory lesions are influential factors of PET/CT imaging.

5. Conclusion

Some of the pulmonary inflammatory lesions with F-18 FDG uptake can cause false positive results of PET tumor imaging. In pulmonary inflammatory lesions, the expression of Glut-1 and Glut-3 were positively correlated with the uptake of F-18 FDG, and Glut-3 plays a more important role in the PET/CT imaging of pulmonary inflammatory lesions. Furthermore, the F-18 FDG uptake of the pulmonary inflammatory lesions is related to the type, quantity, and degree of activation of the inflammatory cells.

References

[1] Hashimoto Y, Tsujikawa T, Kondo C, et al. Accuracy of PET for diagnosis of solid pulmonary lesions with 18F-FDG uptake below the standardized uptake value of 2.5. J Nucl Med 2006;47:426–31.
[2] Meller J, Sahlmann CO, Scheel AK. 18F-FDG PET and PET/CT in fever of unknown origin. J Nucl Med 2007;48:35–45.
[3] Park SG, Lee JH, Lee WA, et al. Biologic correlation between glucose transporters, hexokinase-II, Ki-67 and FDG uptake in malignant melanoma. Nucl Med Biol 2012;39:1167–72.
[4] Krzeslak A, Wojcik-Krowiranda K, Forma E, et al. Expression of GLUT1 and GLUT3 glucose transporters in endometrial and breast cancers. Pathol Oncol Res 2012;18:721–8.
[5] Ming M, Wang ZG, Li D, et al. The applications of corrected standardized uptake values in the diagnosis of peripheral lung lesions. Medicine (Baltimore) 2015;94:e531.
[6] Goo JM, Im JG, Do KH, et al. Pulmonary tuberculosis evaluated by means of FDG PET: findings in 10 cases. Radiology 2000;216:117–21.

Wang et al. Medicine (2016) 95:48 www.md-journal.com
[7] Simpson IA, Dwyer D, Malide D, et al. The facilitative glucose transporter GLUT3: 20 years of distinction. Am J Physiol Endocrinol Metab 2008;295:E242–53.

[8] Davis SL, Nuernberger EL, Um PK, et al. Noninvasive pulmonary [18F]-2-fluoro-Deoxy-D-glucose positron emission tomography correlates with bactericidal activity of tuberculosis drug treatment. Antimicrob Agents Chemother 2009;53:4879–84.

[9] Pellegrino D, Bonab AA, Dragotakes SC, et al. Inflammation and infection: imaging properties of 18F-FDG-labeled white blood cells versus 18F-FDG. J Nucl Med 2005;46:1522–30.

[10] Mochizuki T, Tsukamoto E, Kuge Y, et al. FDG uptake and glucose transporter subtype expressions in experimental tumor and inflammation models. J Nucl Med 2001;42:1551–5.

[11] Kubota K, Furumoto S, Iwata R, et al. Comparison of 18F-fluoromethylcholine and 2-deoxy-D-glucose in the distribution of tumor and inflammation. Ann Nucl Med 2006;20:527–33.

[12] Rogers S, Macheda ML, Docherty SE, et al. Identification of a novel glucose transporter-like protein-GLUT-12. Am J Physiol Endocrinol Metab 2002;282:E733–8.

[13] Fu Y, Maianu L, Melbert BR, et al. Facilitative glucose transporter gene expression in human lymphocytes, monocytes, and macrophages: a role for GLUT isoforms 1, 3, and 5 in the immune response and foam cell formation. Blood Cells Mol Dis 2004;32:182–90.

[14] Nomori H, Watanabe K, Ohtsuka T, et al. Evaluation of F-18 fluorodeoxyglucose (FDG) PET scanning for pulmonary nodules less than 3cm in diameter, with special reference to the CT images. Lung Cancer 2004;45:19–27.