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Original Article

Assessment of vulnerable atherosclerotic and fibrotic plaques in coronary arteries using ^{68}Ga-DOTATATE PET/CT

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Abstract: Activated macrophages which express somatostatin receptor-2 (SSTR-2) play a vital role in rupture of the vulnerable atherosclerotic plaques, which result in death. ^{68}Ga-DOTATATE binds to somatostatin receptors 2, and therefore, can serve as potential radiotracer to detect atherosclerotic plaques. The purpose of this study was to generate preliminary data with this agent in vulnerable or fibrotic atherosclerotic plaques in the coronary arteries. We evaluated a total of 44 patients with neuroendocrine tumors (NET) who underwent ^{68}Ga-DOTATATE PET/CT. In each subject, 7 segments in the coronary arteries were assessed, maximum SUV values and target-to-background ratios (TBRs) were calculated. The lesions detected by CT (a total of 308) were divided into 3 groups based on the Hounsfield unites (HU), and of which, 131 with HU less than 70 were classified as being normal (Control Group), 129 with HU 71-188 as fibrotic plaques (Group 2), and. 48 lesions with HU more than 188 as atherosclerotic plaques (Group 3). The mean TBR value in the normal group was 1.345 ± 0.58 while the mean TBR value in the fibrotic plaque group was 1.752 ± 1.50 (p 0.0043) and in atherosclerotic plaques group was (2.043 ± 1.76, p<0.0001). There was a significant correlation (p=0.0026) between ^{68}Ga-DOTATATE uptake and the progression to formation of atherosclerotic plaques, based on HU. In patients with neuroendocrine tumors, ^{68}Ga-DOTATATE PET/CT showed significantly increased uptake in the fibrotic and vulnerable atherosclerotic plaques compared to normal coronary arteries suggesting a potential role of this tracer for molecular assessment of coronary artery disease in this population.

Keywords: Atherosclerotic plaques, ^{68}Ga-DOTATATE, somatostatin receptor, cardiovascular risk factors, macrophage

Introduction

Cardiovascular disease is the leading cause of death in the United States and is responsible for almost one third of deaths in men and women worldwide [1, 2]. It is estimated that 61 million people in the United States have cardiovascular disease [3]. More than 1 million Americans and more than 19 million individuals worldwide experience a cardiac event every year [4]. Rupture of a vulnerable atherosclerotic plaque is the major cause of cardiac event, which may lead to sudden death [5]. A vulnerable plaque has large lipid core and thin fibrous cap infiltrated by macrophages. The accumulation of activated macrophages in the vulnerable atherosclerotic plaque eventually leads to the ruptures of plaques [6]. Metalloproteinases which are secreted by activated macrophages, will eventually rupture the fibrous cap leading to cardiac event [7, 8]. Several methods have been used to evaluate the atherosclerotic plaques including ultrasonography, CT, MRI, or angiography [9-11], but their role in detecting early disease and response to treatment is limited.

As activated macrophages play an important role in forming and eventually rupturing of the vulnerable atherosclerotic plaques, imaging macrophages may provide a potential means for detecting and characterizing vulnerable ath-

Table 1. Population Demographic

1. No. of Patients	44
2. Sex	Male 23 (52.27%), Female 21 (47.73%)
3. Age (years)	59.84 (± 11.95)
4. Weight (lbs.)	175.91 (± 44.04)
5. Height (inch)	67.80 (± 3.45)
Risk Factors	(%)
1. Prior Vascular Events	9.1
2. Prior CABAG or Stent	9.1
3. Angina	6.8
4. Hypertension	43.2
5. Hyperlipidemia	34.1
6. Smoking	22.7
7. Family History of Hearth diseases	25.0

erosclerotic plaques. It has been proven that activated macrophages overexpress somatostatin receptor 2 (SSTR-2) [13-15]. By now, it has been shown that ^{68}Ga-DOTATATE has affinity for somatostatin receptors, including SSTR-2 and can be successfully used to image neuroendocrine tumors (NET). Therefore, this molecular imaging agent is being adopted as an effective tracer in clinical management for NET in multiple centers in US and EU [16-19]. Several studies have investigated the role of ^{68}Ga-DOTATATE PET/CT in assessment of macrophages and a potential tracer to detect inflammatory process [20-22].

The purpose of this study was to assess the vulnerable atherosclerotic and fibrotic plaques in coronary arteries using ^{68}Ga-DOTATATE PET/CT. We also examined the correlation between ^{68}Ga-DOTATATE uptake and known cardiovascular risk factors in a population of patients with NET.

Methods

Study population

We retrospectively evaluated a total of 308 lesions in the coronary arteries in the left main, LAD, LCX and RCA of the 44 patients with NET who underwent ^{68}Ga-DOTATATE PET/CT from June 2013 through January 2014. The patients were examined with ^{68}Ga-DOTATATE as an Investigational New Drug (IND 117289) approved by the U.S. Food and Drug Administration (FDA), and BRANY Investigational Review Board (IRB). This study was also approved by the institutional review board and all subject signed an informed consent form before they were enrolled into this research study. All patients were histopathology proven with NET based on World Health Organization criteria for cancer [23]. The primary tumor sites were midgut, hindgut, bronchus and pancreas. The entire procedure was explained in detail to the patients and informed consent was obtained. None of the patients received steroids or had a recent history of inflammation or vasculitis. The cardiovascular risk factors including, hypertension, hyperlipidemia, smoking and diabetes were recorded for each subject. Also Body Mass Index (BMI) and history of prior cardiovascular events were included in the data generated.

Patient population

For this analysis, we reviewed clinical and imaging data of 44 patients of whom 23 were males (52%) and 21 were females (48%). All patients were proven to have neuroendocrine cancer by biopsy. Relevant baseline characteristics of the patients and detailed information regarding cardiovascular risk factors are reported in **Table 1**. ^{68}Ga-DOTATATE uptake measurements in the coronary arteries proved to be feasible in all patients (100%). The lesions detected by CT (a total of 308) were divided into 3 groups based on the Hounsfield unites (HU), and of which, 131 with HU less than 70 were classified as being normal (Control Group), 129 with HU 71-188 as fibrotic plaques (Group 2), and. 48 lesions with HU more than188 as atherosclerotic plaques (Group 3).

Image acquisition

For ^{68}Ga-DOTATATE PET, non gated images were acquired 60-90 minutes after injection of 130–185 MBq (4-8mCi) of ^{68}Ga-DOTATATE. PET Imaging were acquired from the top of the head to mid thighs, using a standard PET/CT scanner (Biograph 16, Siemens, Knoxville, TN, USA) in a 3-dimensional mode (3 min per bed position) over an approximate 25-min scanning time. Low-dose CT without intravenous contrast was utilized for anatomic correlation and attenuation correction. The CT kVp and mA exposure factors were determined based on the patient's

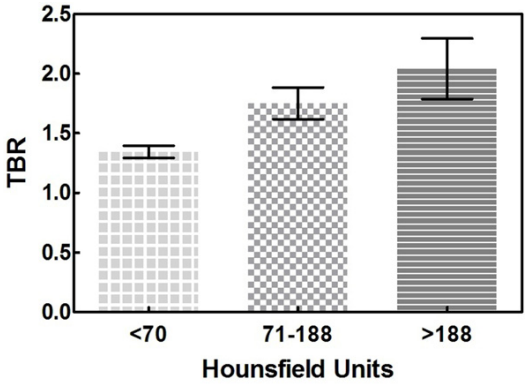

Figure 1. All lesions were divided into 3 groups based on their Hounsfield Units (HU). 130 target areas were classified in group 1 with HU <70 as normal. 129 lesions were categorized in group 2 with HU 71-188 as fibrotic plaques and 48 lesions in group 3 with HU >188 were defined as atherosclotic plaques. The mean uptake value in group I, 2 and 3 was 1.345, 1.751, and 2.043 respectively which proved to be statistically significant between group 1 and 2 (p=0.0043) and group 1 and 3 (p<0.0001).

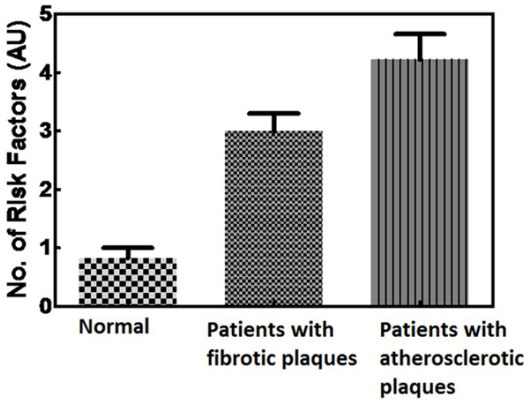

Figure 2. All patients were divided in 3 groups based on their lesion's characteristics. The mean number of risk factors for normal, fibrotic and atherosclerotic plaque groups were 0.83, 3 and 4.22 respectively. One-way analysis of variance was performed in 3 groups and showed statistically significant correlation with the risk factors (p<0001).

body habitus. Patients on long-acting somatostatin analogues were requested to cease these medications for at least 4 weeks prior to initiation of ^{68}Ga-DOTATATE PET/CT imaging.

The ^{68}Ge/^{68}Ga generator (ITGmBH, Germany) was eluted with HCl by fractionated elution. The 2 ml fraction with highest activity (8-22mCi) was added to the clinical kit of DOTATATE. The radiolabeling yield of reaction (%RCY) was determined by Instant Thin Layer Chromatography (iTLC) for QC validation after the final dose formulation. The activity of final ^{68}Ga-DOTATATE dose administered to the patients was in the range of 4-8mCi.

Interpretation of data

In each patient one segment in the left main and 2 proximal segments in each coronary artery including the left coronary artery, right coronary artery and left anterior descending artery were selected as target areas for the purposes of this research study. Arterial calcifications were also assessed; vascular attenuation of >188 Hounsfield units was rated as calcified plaque and that of 77-188 was categorized as representing fibroid plaque [24, 25].

For PET data analysis, a region of interest (ROI)-based approach was chosen. Maximal 'standardized uptake values' (SUVmax) for ^{68}Ga-DOTATATE uptake was calculated for the segments described above. ROIs were placed in the segments noted above in coronary arteries and the Maximum SUV value was extracted for the final calculation of target-to-background ratios (TBRs). Background was defined as the average blood-pool uptake as determined by the mean SUV of two different ROIs (diameter of 1 cm) within the left ventricle. The SUVmax was divided by the mean blood-pool SUV in order to determine the TBR. We used the TBRs of all seven arterial segments for the final analysis. In each of these segments TBR value was correlated with Hounsfield units. We also correlated the uptake value and cardiovascular risk factor in all patients. For ROI assignment, visualization of PET/CT images, and SUV calculation, dedicated software (MIM Software Inc) was used.

Statistical analysis

Statistical analysis was performed using Prism (version 5.00; GraphPad Software) for Windows (Microsoft). Continuous variables with a normal distribution were recorded as mean ± standard deviation. An unpaired t-test was performed between the specified groups. Pearson correlation coefficients were used for the assessment association between the uptake of the tracer (TBR) and the following cardiovascular risk factors: hypercholesterolemia, hypertension, smo-

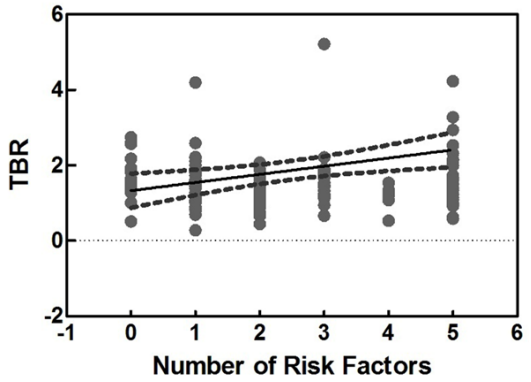

Figure 3. Correlation between the numbers of risk factors and target it background ration of all lesions. The analysis using Pearson regression showed *P* value to be statistically significant (p=0.0068).

king, diabetes, family history, history of cardiovascular disease, age, and gender, as well as BMI.

Results

Atherosclerotic and fibrotic plaques uptake

The mean TBR value was statistically higher in the fibrotic and atherosclerotic plaques groups compared to the normal group as shown in **Figure 1**. The mean TBR value in the normal group was 1.345 ± 0.58 while the mean TBR value in the fibrotic plaque group was 1.752 ± 1.50 (p 0.0043). The atherosclerotic plaques group demonstrated higher TBR compared to the normal group (2.043 ± 1.76, p<0.0001) (**Figure 1**). We noted that fibrotic plaques, which are considered representing an early stage of atherosclerotic plaque formation, also showed higher uptake than normal group. There was a statistically significant correlation (p=0.0026) between ^{68}Ga-DOTATATE uptake with the progression to formation of atherosclerotic plaques, based on HU.

Correlation with risk factors

Atherosclerotic plaques are observed more in individuals with certain risk factors such as hypertension, hyperlipidemia, smoking, etc. To confirm whether the risk factors in the population examined correlated with the TBR values generated, we analyzed our data accordingly. The most common risk factors in our patient population were hypertension, hyperlipidemia, smoking and family history of heart disease. **Figure 2** shows the correlation between ^{68}Ga-DOTATATE TBR mean values and the numbers of cardiovascular risk factors. The mean number of risk factors in the normal group was 0.83, while the mean number of risk factors increased to 3 in fibrotic plaque group, and to 4.22 in atherosclerotic plaque group. One way analysis of variance was performed among these 3 groups and showed a statistically significant *P* value (p<0.0001). **Figure 3** shows correlation between the number of risk factors and target to background ratio of all lesions. We noticed that the patients with higher risk factors demonstrate higher ^{68}Ga-DOTATATE uptake with TBR as demonstrated by rising curve in the **Figure 3**. The pearson regression showed this correlation to be significant (p=0.0068).

Discussion

This research study aimed assessing the vulnerable atherosclerotic and fibrotic plaques in coronary arteries by using ^{68}Ga-DOTATATE PET/CT imaging. We also examined the correlation between ^{68}Ga-DOTATATE uptake and the known cardiovascular risk factors in this population.

^{68}Ga-DOTATATE has also been shown to identify activated macrophages due to overexpression of the SSTR-2 [20]. SSTR-2 expression is upregulated in macrophages that migrate to the inflamed sites in the arteries while the normal endothelial cells lack these receptors.

We noted that there is higher uptake of ^{68}Ga-DOTATATE in atherosclerotic and fibrotic plaques in the coronary arteries likely due to the presence of activated macrophages in the plaques that express SSTR-2 receptors as seen in **Figure 4**. Li et al investigated the role ^{68}Ga-DOTATATE PET/CT for the detection of inflammation in the large arteries [21]. They found strong association of increased ^{68}Ga-DOTATATE uptake with known risk factors of cardiovascular disease in large arteries. Rominger et al. also found a significant correlation between the presence of TBR and calcified plaque in the left anterior descending artery using ^{68}Ga-DOTATATE PET/CT [22]. They also noted that the cut-off TBR value of greater than or equal to 1.5 gave an optimal threshold for specifically distinguishing between patients with and without coronary calcifications.

In our study in the high-risk group patients with higher risk factors, we found the value for TBR for ^{68}Ga-DOTATATE to be significantly higher

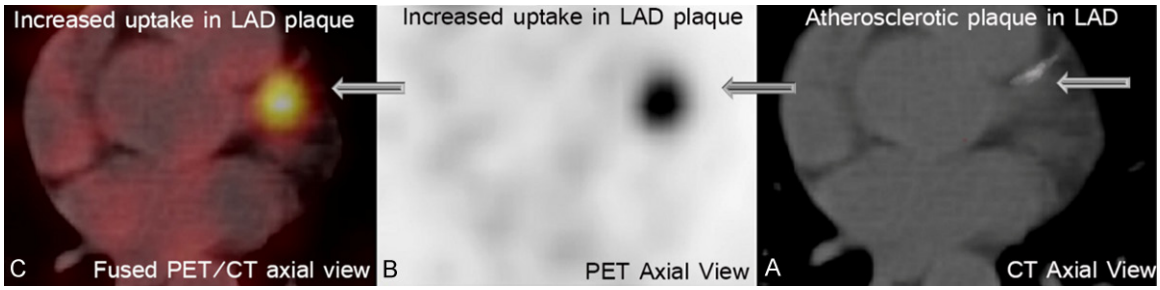

Figure 4. A. Calcified atherosclotic plaque is seen in the proximal left anterior descending coronary artery on axial CT image. B. PET axial image demonstrates high ^{68}Ga-DOTATATE uptake in the same resion in the proximal left anterior descending coronary artery. The increased activity in this atherosclotic plaque is consistent with accumulation of the macrophages in this coronary plaque which suggest vulnerability of this plaque. C. Fused PET/CT axial image shows high ^{68}Ga-DOTATATE uptake in the proximal left anterior descending coronary artery.

than those of patients with lower risk factors. Similar finding was observed in the study by Li et al. in which ^{68}Ga-DOTATATE uptake correlated significantly with hypertension, age, and the presence of calcifications. They demonstrated that the risk factors for CAD correlated less strongly with foci of increased ^{18}F-FDG uptake as compared to those of ^{68}Ga-DOTATATE. This might be interpreted as an indication that ^{68}Ga-DOTATATE is more specific for the inflamed plaque.

It has also been demonstrated the SSTR-2 also plays a role in atherosclerotic plaques due to presence of angiogenesis which has been described in the context of unstable plaques [26]. A study by Adams et al. demonstrated a correlation between proliferation of the human umbilical vein endothelial cells and increased expression of SSTR-2 receptors, which suggests an active role for this receptor in angiogenesis. Therefore, ^{68}Ga-DOTATATE PET/CT may be able to assess unstable plaques due to two different mechanisms: detection of inflammation as well as angiogenesis within the atherosclerotic lesion.

The limitations of our study are the small sample size, non-gated image acquisition and lack of histological validation of ^{68}Ga-DOTATATE uptake. Also, this study was performed in patients with cancer, and therefore, our findings may be not generalized to a study population of patients with well characterized coronary artery disease.

Conclusions

Our study demonstrate that in a series of patients with NET and atherosclerotic and fibrotic plaques in coronary arteries, ^{68}Ga-DOTATATE PET/CT shows significantly increased uptake compared to normal coronary arteries suggesting a potential role for this tracer in patients with coronary artery disease. Further studies are needed to evaluate the role of ^{68}Ga-DOTATATE PET/CT imaging in atherosclerosis.

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