Imaging the Cytokine Receptor CXCR4 in Atherosclerotic Plaques with the Radiotracer $^{68}$Ga-Pentixafor for PET

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$^{68}$Ga-pentixafor is a radiotracer for PET that binds with nanomolar affinity to CXCR4. The CXCR4 receptor is expressed at the surface of inflammatory cells. The objective of the study was to analyze the ability of radiolabeled pentixafor to detect CXCR4 expression on inflammatory cells present in atherosclerotic plaques of an experimental rabbit model. Methods: Atherosclerotic plaques were induced by endothelial abrasion of the right carotid artery and abdominal aorta of 7 rabbits fed an atherogenic diet. Five noninjured rabbits fed a chow diet were used as controls. Rabbits were imaged on a PET/MR system after injection of $^{68}$Ga-pentixafor (15 MBq/kg). Vascular signal was quantified as tissue-to-background ratio (TBR). Biodistribution and autoradiographic studies were performed 1 hour after injection of $^{125}$I-pentixafor (7.5 MBq/kg). In addition, blocking studies were performed in 2 atherosclerotic rabbits with preinjection of the CXCR4 inhibitor AMD3100. Tracer uptake was quantified on arterial cryosections using autoradiography and compared with CXCR4 and RAM-11 (macrophage) expression on adjacent histologic sections. Results: One hour after injection of $^{68}$Ga-pentixafor, strong signals were detected in vivo with PET/MR imaging in atherosclerotic plaques of the abdominal aorta and right carotid artery as compared with normal control arteries (mean TBR = 1.95 ± 0.51 vs. 1.22 ± 0.25 and mean TBR = 1.24 ± 0.38 vs. 0.96 ± 0.37, respectively; $P < 0.05$ for both). Blocking studies with preinjection of a CXCR4 inhibitor reduced $^{125}$I-pentixafor uptake in atherosclerotic plaques by approximately 40%. $^{125}$I-pentixafor uptake in the vessel wall on autoradiographies was located in macrophage-rich regions of atherosclerotic plaques and correlated with the intensity of CXCR4 expression on corresponding cryosections ($R^2 = 0.61$; $P < 0.05$). Conclusion: $^{68}$Ga-pentixafor allows for the noninvasive detection of CXCR4 expression in the vessel wall with PET and emerges as a potential alternative to $^{18}$F-FDG for the assessment of macrophage infiltration in atherosclerotic plaques.

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In the past 10 y, $^{18}$F-FDG PET has proved a reliable noninvasive imaging approach to assess the degree of inflammation present in atherosclerotic plaques both in animal models of atherosclerosis and in patients (1,2). Patients with the highest $^{18}$F-FDG uptake in plaques present a higher rate of cardiovascular events and worse prognosis than patients with low vascular $^{18}$F-FDG uptake (3). $^{18}$F-FDG PET detects inflammation with high sensitivity but has important limitations in the specific setting of cardiovascular imaging: physiologic uptake in the heart and in the brain, background signal of blood, interaction with blood glucose, need for a long fasting period, or dedicated diet before imaging (4). Consequently, radiotracers that more specifically target inflammatory cells might greatly facilitate the noninvasive detection of inflammatory activity in the vessel wall with PET.

$^{68}$Ga-pentixafor is the first PET agent that exhibits high affinity and selectivity for C-X-C chemokine receptor type 4 (CXCR4) (5). The CXCR4 is a constitutive cytokine receptor involved in several biologic processes including the entry of HIV, the development of metastasis, and the evolution of different autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis (6). CXCR4 expression has been identified in more than 30 different types of cancers, and its activation is a key trigger for enhanced tumor growth and progression, tumor invasiveness, and metastasis. $^{68}$Ga-pentixafor was therefore first evaluated in several tumors expressing CXCR4 in animal models and demonstrated both high and specific uptake in CXCR4-positive tumors and metastases (7). Proof-of-concept studies have recently been performed with $^{68}$Ga-pentixafor in patients with lymphoma (8) and multiple myeloma (9), both tumor entities expressing high levels of CXCR4, and demonstrated intense uptake in tumor...
cells, excellent pharmacokinetics, and favorable dosimetry in humans (10), with a low residual signal in the blood. Furthermore, CXCR4 plays a pivotal role in the trafficking of inflammatory cells by mediating the homing of progenitor cells in the bone marrow and regulating their mobilization into peripheral tissues on injury (6). In fact, the small molecular antagonist AMD3100 (plerixafor) blocks the CXCR4 receptor and allows for mobilization of hematopoietic stem and progenitor cells in the bone marrow for autografting on myeloablative treatment (11). Because of the overexpression of CXCR4 at the surface of cells involved in the inflammatory process, we hypothesized in this study that 68Ga-pentixafor might find a role in the detection of inflammatory cells with PET. Moreover, CXCR4 expression is strongly increased under hypoxic conditions through the stimulation of the hypoxia-inducible factor (12). 68Ga-pentixafor therefore represents a promising candidate for the detection of high-risk atherosclerotic plaques, which contain intense inflammatory reaction and hypoxic conditions.

The aim of this study was to test whether 68Ga-pentixafor specifically binds to CXCR4-expressing tissues and can be detected in atherosclerotic plaques induced in the abdominal aorta and right carotid artery of rabbits. In addition, we assessed the expression of CXCR4 in carotid plaques in a small group of patients with 68Ga-pentixafor PET imaging and on histologic sections to evaluate whether the results observed in a rabbit model with 68Ga-pentixafor may translate in the evaluation of human atherosclerotic plaques.

MATERIALS AND METHODS

Radiolabeling of Pentixafor

Radiosynthesis of 68Ga-Pentixafor. 68Ga was obtained by elution of a 68Ge/68Ga generator with SnO2 matrix (iTHEMBA LABS) with 1 M HCl (5.5 mL) and immobilized on a strong cationic exchanger cartridge (SCX-Chromafix, size M; Macherey-Nagel).

For animal studies, 68Ga-pentixafor was prepared on a Gallelut+ system in analogy to a previously published 68Ga-labeling procedure (SCINTOMICS GmbH) (13). Briefly, 68Ga generator eluate fractions (1.25 mL, 600–800 MBq, buffered to pH 3.3 with 900 μL of a solution of 14.4 g N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) in 12 mL of water) were reacted with 3.5 nmol of pentixafor (SCINTOMICS GmbH) for 5 min. The radiochemical purity was always greater than 99% as confirmed by thin-layer chromatography and high-performance liquid chromatography (HPLC). Addition of 1 mL of phosphate-buffered saline and concentration in vacuo to 1 mL of total volume yielded solvent-free formulations with specific activities ranging from 100 to 150 MBq/μmol.

For patient studies, the synthesis of 68Ga-pentixafor was performed in a fully automated, good manufacturing practices–compliant procedure using a GRP module (SCINTOMICS GmbH) equipped with a disposable single-use cassette kit (ABX) including a standardized labeling sequence (14). Upon being washed with water, 68Ga3+ was eluted into the reaction vessel (containing 20 μg of pentixafor in 3 mL of 1.5 M N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) using 1.7 mL of 5 M NaCl). The reaction mixture was heated to 140°C for 10 min and, upon cooling, transferred onto a 18 light cartridge (Waters). The cartridge was washed with water to remove unreacted 68Ga activity, and 68Ga-pentixafor was eluted using 2 mL of EtOH/water (50/50). The eluate was diluted with 12 mL of phosphate-buffered saline and passed through a sterile filter. The radiochemical purity of the ready-to-inject formulation (14 mL) was always greater than 99% as confirmed by radio-HPLC and thin-layer chromatography, and the specific activity was in the range of 30–65 GBq/μmol.
PET/MR Imaging Protocols. Imaging was performed on a human whole-body simultaneous PET/MR (3T) scanner (Biograph mMR; Siemens). Rabbits were anesthetized with an intravenous infusion of Propofol (2%, −1.2–1.3 mg/kg/min; Propofol 2%; MCT Fresenius, Fresenius Kabi Deutschland GmbH) and intubated, and catheters were placed in ear veins. Rabbits positioned prone in the scanner were ventilated (Servo-I; Maquet Critical Care AB) and monitored (pulse oxymeter and electrocardiogram) during the whole imaging session. PET and MR images were acquired simultaneously in all rabbits and started 45 min after injection of 15 MBq/kg of 68Ga-pentixafor. Detailed PET and MR acquisition protocols are provided in the section “PET and MR Acquisition Protocols.”

Image Analysis. Images were analyzed by an experienced nuclear medicine physician masked to the rabbit group and to the results of histology. Image registration and fusion of the time-of-flight MR acquisitions and attenuation-corrected PET images were performed with an OsiriX workstation (OsiriX version 3.5.1 64-bit; OsiriX Imaging Software), an open-source DICOM viewer and image manipulator using the Horn algorithm, a manual rigid registration algorithm based on multiple points (16,17). Matching of both datasets was considered as correct when rabbit contours of PET and MR images were perfectly aligned. If required (e.g., in the case of animal motion), alignment was corrected manually using the Fusion tool of the OsiriX software. For biodistribution studies, SUVmean calculated as decay-corrected tissue radioactivity divided by body weight and injected dose was measured in circular regions of interest placed on each organ located using the MR images.

For the quantification of the vascular uptake, circular regions of interest were placed on axial adjacent PET images of the abdominal aorta and both carotid arteries (12 axial sections each) identified using the time-of-flight images. SUVmax was recorded in each region of interest. In addition, the SUVmean of blood was measured by placing a circular region of interest in the right atria. The tissue-to-background ratio (TBR) was calculated as the ratio of SUVmax and the background venous activity measured as SUVmean of blood (18). Mean TBRs were calculated for each arterial territory as the average of TBR values throughout the entire arterial segment.

PET and MR Acquisition Protocols
MR Acquisition Protocol. MR imaging was performed using a clinical knee coil for the aorta and a bilateral 4-channel phased array coil for carotid arteries (4-channel, Machnet BV). MR acquisition first started with a localizer scan to define the bed position and was followed by a time-of-flight 3-dimensional angiographic sequence to visualize the abdominal aorta. Subsequently, a coronal 2-point Dixon 3-dimensional volumetric interpolated breath-hold T1-weighted (VIBE) MRI sequence was acquired for the generation of attenuation maps (μ-maps) and anatomic images in association with the PET acquisition. The Dixon MRI sequence was set as follows: integrated parallel acquisition technique; acceleration factor, 2; voxel size, 3.7 × 2.3 × 2.7 mm³ (in-plane resolution × slice thickness); acquisition time, 19 s; repetition time, 3.6 ms; first echo time, 1.23 ms; second echo time, 2.46 ms; matrix, 192 × 192; number of excitations, 1; field of view, 450 mm; phase field of view, 65.6%; 1 slab with 128 slices; slice thickness, 2.73 mm; flip angle, 10³; and bandwidth, 965 Hz/pixel. The software of the MRI scanner automatically used the raw images to generate 4 different images: T1-weighted in-phase, T1-weighted out-of-phase, water-only, and fat-only. The same MR acquisition protocol was repeated for the imaging of carotid arteries.

PET Acquisition Protocol. Static emission scans were acquired during 10 min in a single bed position 50 min after the injection of 68Ga-pentixafor in the abdominal aorta and 60 min after the injection for carotid arteries. Data were corrected for randoms, dead time, scatter, and attenuation based on the μ-maps generated from the Dixon images (19). Two-mm-thick axial PET images were reconstructed with a 50 × 50 cm² field of view and a 344 × 344 matrix using a 3-dimensional attenuation-weighted ordered-subsets expectation maximization iterative reconstruction algorithm with 3 iterations and 21 subsets, a zoom of 1, and no postreconstruction filter. Spatial resolution of the reconstructed PET images was previously determined to be 4.3 mm at 1 cm from the scanner’s isocenter and 5.0 mm at 10 cm in the transverse direction in full width at half maximum (20).

Tracer Biodistribution and Histology
Tracer Biodistribution in Tissues. At the end of the imaging session, 8 rabbits (atherosclerotic rabbits, n = 5; control rabbits, n = 3) were injected intravenously with 7.5 MBq/kg of 125I-pentixafor into the ear vein under isoflurane anaesthesia for biodistribution studies. Because of the long half-life of 125I (60 d), 125I-pentixafor allowed for more accurate biodistribution studies and autoradiographies in comparison to 68Ga-pentixafor (half-life, 68 min). The animals were sacrificed 60 min after injection by intravenous injection of 150 mg/kg of pentobarbiturate.

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Statistical Analysis

Numeric values are expressed as mean ± SD. Statistical analysis was performed using SPSS software (SPSS Inc.). Comparisons of quantitative values measured in 2 groups were performed using a 2-tailed Student t test if the number of values was greater than 20 in each group and using a Wilcoxon signed-rank test if the number of values was less than 20 in each group. For fluorescence-activated cell sorting
Known to express physiologically CXCR4 receptors (Fig. 2). The tracer. The specificity of 68Ga-pentixafor uptake in the spleen (SUVmean = 1.7 ± 0.3 vs. 0.7 ± 0.2 for the spleen and SUVmean = 1.9 ± 0.7 vs. 0.5 ± 0.1 for adrenal glands, P < 0.05 for both; Fig. 3). In addition, focal areas of 68Ga-pentixafor uptake were detected in the abdominal aortas and right carotid artery of atherosclerotic rabbits by PET imaging, whereas no significant uptake was detected in the aortas and carotid arteries of atherosclerotic rabbits as well as in control rabbits after blocking the CXCR4 receptor. 68Ga-pentixafor uptake in atherosclerotic plaques was quantified in vivo as the average TBR over the different arterial segments (Fig. 3). Higher TBRs were measured in the abdominal aorta and right carotid artery as compared with normal control arteries (mean TBR = 1.95 ± 0.51 vs. 1.22 ± 0.25 and mean TBR = 1.24 ± 0.38 vs. 0.96 ± 0.37; P < 0.05 for both). TBRs measured in the abdominal aorta and in the right carotid artery were significantly reduced (~40%) after blocking CXCR4 receptors (mean TBR = 1.95 ± 0.51 vs. 1.13 ± 0.52 and mean TBR = 1.24 ± 0.38 vs. 0.70 ± 0.03; P < 0.05 for both).

Biodistribution of Radiolabeled Pentixafor in Rabbits. The percentage injected activity adjusted for weight (%IA/g) was quantified 1 h after the injection of 125I-pentixafor in the different organs with and without blocking CXCR4 receptors (Fig. 3). Biological studies confirmed the binding of radiolabeled pentixafor analysis, the levels of CXCR4 expression in the different groups were compared with a Kruskal–Wallis test followed by a Dunn multiple comparison test. Pentixafor uptake on autoradiography and CXCR4 expression on corresponding sections were compared using multiple regression to take into account that 3 samples were analyzed in each animal. P values of less than 0.05 were considered as significant.

RESULTS

Detection of CXCR4 with 68Ga-Pentixafor in Rabbit Model of Atherosclerotic Plaques

In Vivo Imaging of Atherosclerotic Plaques with 68Ga-Pentixafor PET/MRI. Fifty minutes after intravenous injection of 68Ga-pentixafor, the radiotracer accumulated in the spleen (SUVmean = 1.7 ± 0.3) and in adrenal glands (SUVmean = 1.9 ± 0.7), organs known to express physiologically CXCR4 receptors (Fig. 2). The highest activity was located in the kidneys (SUVmean = 3.2 ± 0.5), which reflects the renal-mediated clearance of the radiotracer. The specificity of 68Ga-pentixafor uptake in the spleen and in adrenal glands was confirmed by blocking CXCR4 recep-

tors with a preinjection of AMD3100, which significantly reduced the signal in these organs (SUVmean = 1.7 ± 0.3 vs. 0.7 ± 0.2 for the spleen and SUVmean = 1.9 ± 0.7 vs. 0.5 ± 0.1 for adrenal glands, P < 0.05 for both; Fig. 3). In addition, focal areas of 68Ga-pentixafor uptake were detected in the abdominal aortas and right carotid artery of atherosclerotic rabbits by PET imaging, whereas no significant uptake was detected in the aortas and carotid arteries of atherosclerotic rabbits as well as in control rabbits after blocking the CXCR4 receptor. 68Ga-pentixafor uptake in atherosclerotic plaques was quantified in vivo as the average TBR over the different arterial segments (Fig. 3). Higher TBRs were measured in the abdominal aorta and right carotid artery as compared with normal control arteries (mean TBR = 1.95 ± 0.51 vs. 1.22 ± 0.25 and mean TBR = 1.24 ± 0.38 vs. 0.96 ± 0.37; P < 0.05 for both). TBRs measured in the abdominal aorta and in the right carotid artery were significantly reduced (~40%) after blocking CXCR4 receptors (mean TBR = 1.95 ± 0.51 vs. 1.13 ± 0.52 and mean TBR = 1.24 ± 0.38 vs. 0.70 ± 0.03; P < 0.05 for both).

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in adrenal glands, spleen, and bone marrow, which was significantly reduced in rabbits blocked with a dose of 20 mmol of AMD3100 (≈2,000-fold molar excess over pentixafor), an established CXCR4 inhibitor, before injection of 125I-pentixafor (0.33 ± 0.14 vs. 0.02 ± 0.01 %IA/g for adrenal glands; 0.17 ± 0.09 vs. 0.03 ± 0.01 %IA/g for spleen; 0.14 ± 0.04 vs. 0.03 ± 0.01 %IA/g for bone marrow). In addition, significantly higher activities were measured in the right atherosclerotic carotid arteries and abdominal aortas in comparison to the noninjured left carotid arteries and abdominal aortas of control rabbits (0.08 ± 0.02 %IA/g and 0.10 ± 0.03 %IA/g vs. 0.05 ± 0.01 %IA/g and 0.05% ± 0.01 %IA/g, respectively, P < 0.05 for both).

Correlation Between 125I-Pentixafor Accumulation and Expression of CXCR4 in Plaques

CXCR4 expression was mostly located in macrophage-rich (RAM-11–positive) regions of atherosclerotic plaques (Fig. 4). The percentage of CXCR4-positive immunostaining was significantly higher in atherosclerotic plaques of the abdominal aorta and right carotid artery relative to the corresponding segments of control rabbits (75% ± 25% vs. 26% ± 13% and 58% ± 15% vs. 23% ± 16%; P < 0.05 for both; Fig. 5). In addition, atherosclerotic plaque sections that exhibited high-counting rates, as detected by autoradiography, contained high CXCR4 expression in the corresponding adjacent histologic sections (Fig. 4). In contrast, atherosclerotic plaques with low autoradiographic activities as well as normal aortic walls from control rabbits contained only few cells expressing CXCR4. A strong correlation (r² = 0.61; P < 0.05) was found between the mean activity quantified on autoradiography in arterial sections and the percentage of CXCR4-positive areas measured in the arterial wall on adjacent histologic sections by immunohistology (Fig. 5). Interestingly, the addition of oxidized low-density lipoprotein to macrophage cell culture induced a strong increase in the expression of CXCR4 at the cell surface detected with fluorescence-activated cell sorting analysis (Fig. 5; 17,892 ± 5,964 vs. 39,157 ± 22,285; P < 0.05), whereas incubation with tumor necrosis factor α did not have any effect on CXCR4 expression on macrophages (17,892 ± 5,964 vs. 18,200 ± 4,683; P > 0.05). Incubation with statins did not have any effect on CXCR4 expression at the surface of macrophages incubated with oxidized low-density lipoprotein (48,600 ± 6,520 vs. 39,157 ± 22,285; P > 0.05).

Detection of 68Ga-Pentixafor Uptake with PET in Human Carotid Plaques

68Ga-pentixafor uptake in carotid arteries was analyzed with PET/MR imaging in 4 patients with carotid stenosis greater than 50% and 4 patients with carotid stenosis less than 30%. Focal, intense uptake of 68Ga-pentixafor (SUVmax = 3.4 and 2.1, and most-diseased-segment TBR = 2.6 and 2.1) was identified in carotid atherosclerotic plaques of 2 of 4 patients with carotid stenosis greater than 50% (Supplemental Table 1; supplemental materials are available at.

FIGURE 6. In vivo imaging of human carotid atherosclerotic plaques with 68Ga-pentixafor PET/MRI. Representative example of focal accumulation of 68Ga-pentixafor in carotid atherosclerotic plaque detected with PET/MRI. Interestingly, this patient had presented for 2 wk ischemic stroke ipsilateral to carotid plaque showing high 68Ga-pentixafor uptake (white arrows). Note also intense accumulation of 68Ga-pentixafor in perivascular lymph node and in tonsils (white arrowheads). MIP = maximum-intensity projection.

FIGURE 7. Representative examples of CXCR4 expression in human carotid plaques on immunohistochemistry. In human carotid plaques, CXCR4 expression by immunohistochemistry was mostly located in macrophage-rich areas (CD-68–positive cells), whereas only weak expression of CXCR4 was present in lymphocyte-rich areas (CD-45–positive cells) and in endothelial cells (CD-31–positive cells).
In this study, using a rabbit model of atherosclerosis, we demonstrate that the expression of CXCR4 in atherosclerotic plaques can be detected in vivo with PET imaging after injection of 68Ga-pentixafor. A significantly higher uptake of 68Ga-pentixafor was detected both in vivo and ex vivo in atherosclerotic plaques of rabbits compared with normal arterial wall and was significantly reduced after a preinjection of the potent CXCR4 inhibitor AMD3100, excluding a nonspecific retention of the tracer in the arterial wall. In addition, the intensity of 68Ga-pentixafor uptake in the aortic wall quantified on autoradiography strongly correlated with CXCR4-positive areas measured on adjacent histologic sections. Furthermore, we confirmed in a small number of patients that focal 68Ga-pentixafor uptake can be detected in carotid atherosclerotic plaques with PET. In human carotid atherosclerotic plaques, we found that CXCR4 expression was localized in macrophage-rich areas. Hence, 68Ga-pentixafor represents a promising PET radiotracer for identification of macrophage infiltration present in high-risk atherosclerotic plaques.

Targeting CXCR4 in Atherosclerotic Plaques

The precise role of CXCR4 expression in atherosclerosis is complex and remains controversial (6). CXCR4 might play a proatherogenic role in attracting leukocytes in plaques but may also have a protective role through the recruitment of endothelial cells. In addition, CXCR4 expression is modulated by heteromerization with CXCR7 (21) or by interaction with hetero-complexes formed of CXCL12 and high-mobility group box 1 proteins (22), underscoring the limitations of messenger RNA and protein expression measurements of CXCR4 performed so far in atherosclerotic plaques. Radiolabeled pentixafor presents the advantage of binding exclusively to CXCR4 expressed on the cell membrane, and its uptake might therefore give a more precise estimation of the location and number of functional CXCR4 proteins in atherosclerotic plaques. Interestingly, we evidenced in this study a strong correlation between the intensity of radiolabeled pentixafor uptake in plaques and the expression of CXCR4 on immunohistochemistry. Radiolabeled pentixafor might thus provide an interesting opportunity to understand more in depth the functional expression of CXCR4 in plaques and its changes over time using in vivo PET imaging.

Imaging Macrophages in Atherosclerotic Plaques with 68Ga-Pentixafor

To be relevant for the evaluation of atherosclerotic plaques, the biologic target of a radiotracer needs to fulfill several conditions: to be sufficiently expressed in plaques so that the radiotracer can reach concentrations allowing for its detection with PET, to differ significantly between high-risk and stable plaques, and to be associated with the risk of subsequent plaque rupture and clinical events. On the basis of these criteria, macrophages appear as a relevant target for atherosclerosis imaging: high macrophage density can be found in atherosclerotic plaques (23), the number of macrophages is significantly higher in complicated versus uncomplicated plaques (24,25), and the identification of macrophages in atherosclerotic plaques has been associated with an increased risk of cardiovascular events during follow-up (26). Interestingly, we found in this study that radiolabeled pentixafor accumulated in macrophage-rich regions of rabbit and human atherosclerotic plaques. By the means of fluorescence-activated cell sorting analysis, we furthermore confirmed that a low-density lipoprotein-rich milieu induced an increase in the expression of CXCR4 at the surface of macrophages. Hence, 68Ga-pentixafor appears relevant for the evaluation of atherosclerotic plaques as a noninvasive imaging marker of macrophage infiltration in the vessel wall.

Advantages of 68Ga-Pentixafor Over Existing PET Radiotracers in Atherosclerosis Imaging

Over the past 10 y, several PET radiotracers have been developed for the imaging of atherosclerotic plaques, each of them targeting specific biologic activities, cellular types, or protein expression increased in high-risk plaques (27). Nevertheless, 68Ga-pentixafor might hold several advantages over existing PET radiotracers in this indication. First, the intensity of the signal in atherosclerotic plaques was high enough to be detectable with PET. Second, physiologic expression of CXCR4 is limited to the spleen, adrenal glands, and bone marrow. 68Ga-pentixafor uptake in atherosclerotic plaques can thus be precisely assessed thanks to a low background signal in tissues adjacent to the arterial wall (myocardium, brain, muscle). Third, 68Ga-pentixafor-PET imaging does not require the patient to fast as is the case for 18F-FDG. Fourth, pentixafor can readily be radiolabeled with the generator nuclide 68Ga, which is obtained after elution from a 68Ge/68Ga generator and does not require the use of an on-site cyclotron. The flexibility of this synthesis could be particularly interesting for cardiovascular imaging. Taken together, 68Ga-pentixafor might greatly facilitate the noninvasive detection of CXCR4 expression and macrophage infiltration in atherosclerotic plaques with PET.

This study shows minor limitations. First, additional studies are required to unravel in more details the cellular processes in which CXCR4 is involved and the underlying molecular mechanisms associated with its expression in atherosclerotic plaques. In this context, the understanding of the complex role of CXCR4 will be significantly improved by the availability of an imaging method allowing for the in vivo monitoring of CXCR4 receptor expression in atherosclerotic plaques. Of note, radiolabeled pentixafor shows only a low affinity for murine CXCR4 and can therefore not be used to follow CXCR4 expression over time in an atherosclerotic mice model. Second, the level of CXCR4 expression on macrophages measured using fluorescence-activated cell sorting analysis was not significantly affected by ex vivo incubation with statins. Additional experiments are, however, required to test the effects of systemic lipid-lowering on CXCR4 expression at the surface of macrophages. Third, definite conclusions cannot be drawn on the value of 68Ga-pentixafor PET imaging for plaque imaging because the number of patients evaluated in this study was small. In a forthcoming study, we plan to evaluate in 45 additional patients with high-grade carotid stenosis the interest of 68Ga-pentixafor
PET imaging for the detection of CXCR4 expression in plaques and to compare the intensities of 68Ga-pentixafor and 18F-FDG uptake in human plaques. The results of this clinical study will give us more hints on the value of 68Ga-pentixafor PET imaging for the evaluation of atherosclerotic plaques. Finally, whether the intensity of 68Ga-pentixafor uptake detected with PET in the vascular wall represents a relevant biomarker to predict the risk of plaque rupture and subsequent clinical events will need to be validated in future studies.

**CONCLUSION**

In this study, we demonstrated that the intensity of CXCR4 expression in atherosclerotic plaques could be assessed with 68Ga-pentixafor PET imaging in a rabbit model. In addition, we have confirmed in a small number of patients that 68Ga-pentixafor uptake can be detected in human carotid plaques. Taken together, these results support a potential role of 68Ga-pentixafor PET imaging for the more specific identification of macrophages in atherosclerotic plaques by overcoming the current limitations of 18F-FDG for the detection of inflammation in the cardiovascular field. Further studies will need to be performed to understand more in depth the origin, meaning, and changes of CXCR4 expression in tissues taking place during inflammatory processes. In fact, the interest of 68Ga-pentixafor PET imaging is not restricted to atherosclerosis imaging but might also find a role for the more specific detection of inflammatory cells in organs with intrinsic high 18F-FDG uptake such as the heart.

**DISCLOSURE**

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