18-kDa Translocator Protein Ligand ^{18}F-FEMPA: Biodistribution and Uptake into Atherosclerotic Plaques in Mice

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ABSTRACT

Background: Radioligands of 18-kDa translocator protein (TSPO) expressed on activated macrophages are a potential approach for imaging of inflammation in atherosclerosis. We evaluated a novel TSPO-targeted tracer ^{18}F-FEMPA for the detection of atherosclerotic plaque inflammation in mice. **Methods and results:** The distribution kinetics of ^{18}F-FEMPA was evaluated by *in vivo* PET/CT imaging. ^{18}F-FEMPA uptake was compared in atherosclerotic (LDLR$^{-/-}$ ApoB$^{100/100}$, *n*=10) and healthy mice (C57BL/6N, *n*=7) *ex vivo* at twenty minutes post-injection. Biodistribution was analyzed from harvested tissue samples, and aortas were sectioned for autoradiography. Aortas of LDLR$^{-/-}$ApoB$^{100/100}$ mice showed large, macrophage-rich atherosclerotic plaques. *In vivo*, ^{18}F-FEMPA showed rapid blood clearance but no difference in aortic uptake between atherosclerotic and healthy mice. In the mice studied *ex vivo* at 20 minutes post-injection, quantification of radioactivity in the whole aorta showed 1.3-fold higher^{18}F-FEMPA accumulation in atherosclerotic than healthy mice (*P*=.028). Autoradiography showed higher tracer uptake in plaque areas with high macrophage content as compared with areas of no macrophages (count densities 190±54 vs. 40±13 PSL/mm^2, *P*<.001*)*, but the uptake in the plaques was not higher than in the normal vessel wall (230±78 PSL/mm^2). *In vitro* blocking showed specific accumulation in mouse and human atherosclerotic plaques. Immunohistochemistry confirmed co-localization of TSPO and macrophages. **Conclusions:** ^{18}F-FEMPA shows rapid blood clearance and uptake in the mouse aorta. Uptake in atherosclerotic plaques correlated with the amount of macrophages, but did not exceed that in the normal vessel wall.

Key Words: atherosclerosis; ^{18}F-FEMPA; inflammation; PET/CT imaging; 18-kDa translocator protein

Abbreviations:

^{11}C-PK11195	*N*-methyl-^{11}C-(*R*)-1-(2-chlorophenyl)-*N*-(1-methyl-propyl)-3-isoquinoline carboxamide
^{18}F-FDG	2-^{18}F-fluoro-2-deoxy-*D*-glucose
^{18}F-FEMPA	*N*-{2-[2-^{18}F-fluoroethoxy]-5-methoxybenzyl}-*N*-[2-(4-methoxyphenoxy)pyridin-3-yl]acetamide
α-SMA	Alpha-smooth muscle actin
H&E	Hematoxylin and eosin
PET/CT	Positron emission tomography/computed tomography
PSL/mm^2	Photo-stimulated luminescence per square millimetre
ROI	Region of interest
SUV	Standardized uptake value
TSPO	Translocator protein (18-kDa)
VSMCs	Vascular smooth muscle cells

INTRODUCTION

Inflammation of the vessel wall plays a role in the development of atherosclerosis and its complications[1,2]. Circulating monocytes are recruited to plaques where they differentiate into macrophages and cholesterol-filled foam cells. Macrophages secrete inflammatory cytokines and produce proteolytic enzymes that can weaken the protective fibrous cap of the plaque and thus, make it vulnerable to rupture. Therefore, molecular imaging of vascular inflammation may provide a risk marker of future vascular events and a surrogate marker of therapy responses[2,3].

Positron emission tomography/computed tomography (PET/CT) imaging with the glucose analogue 2-^{18}F-fluoro-2-deoxy-*D*-glucose (^{18}F-FDG) has been reported to detect inflammation in the arterial wall[2]. However, there is a need for more specific tracers for atherosclerotic plaque inflammation than ^{18}F-FDG[4]. The uptake of ^{18}F-FDG in macrophages and the corresponding PET signal in atherosclerotic plaques may be influenced by the availability of exogenous glucose[2], activation state of vascular cells[5], and hypoxia[6]. Furthermore, ^{18}F-FDG shows high physiological uptake in the myocardium, which is a disadvantage for coronary artery imaging.

18-kDa translocator protein (TSPO) is ubiquitously expressed and localized primarily in the outer mitochondrial membrane. It is involved in numerous cellular functions, such as steroid hormone synthesis and cholesterol transport. TSPO may also be involved in atherosclerosis development, making it a possible drug target[7,8,9]. The expression of TSPO is high in activated macrophages[10,11] and therefore, it has been targeted for imaging inflammatory conditions[3,12]. Since macrophages are the main inflammatory cell type in atherosclerotic plaques, TSPO ligands could be suitable for detection of inflammation associated with atherosclerosis[3].

Previously, TSPO ligand *N*-methyl-^{11}C-(*R*)-1-(2-chlorophenyl)-*N*-(1-methyl-propyl)-3-isoquinoline carboxamide (^{11}C-PK11195) has been tested for imaging atherosclerosis. *In vitro* studies have shown ^{11}C-PK11195 binding to macrophage-rich human atherosclerotic plaques[3,13]. *In vivo* imaging showed uptake of ^{11}C-PK11195 in carotid artery plaques of symptomatic patients, but target-to-background ratio was low[14]. Due to the often low signal-to-noise ratio for *in vivo* imaging with ^{11}C-PK11195, new TSPO targeting tracers have been developed[11,12]. Fluorine-18-FEMPA (BAY 1006578**,** *N*-{2-[2-^{18}F-fluoroethoxy]-5-methoxybenzyl}-*N*-[2-(4-

methoxyphenoxy)pyridin-3-yl]acetamide, CAS 1207345-42-3) (^{18}F-FEMPA) is a second-generation TSPO ligand for PET imaging[15]. It has been studied in the imaging of neuroinflammation, whereas the potential for the detection of atherosclerotic plaque inflammation has not been evaluated yet. Provided that ^{18}F-FEMPA shows uptake in atherosclerotic plaques, the ideal physical properties of ^{18}F radionuclide make it an attractive tracer for *in vivo* PET imaging.

The purpose of this study was to evaluate the feasibility of ^{18}F-FEMPA imaging for the detection of atherosclerotic plaque inflammation in a mouse model. *In vivo* PET/CT imaging and *ex vivo* biodistribution studies were utilized to assess the whole-body distribution and kinetics of ^{18}F-FEMPA. The uptake of ^{18}F-FEMPA in the atherosclerotic plaques of hypercholesterolemic mice was measured by autoradiography and compared with the degree of macrophage infiltration. The uptake specificity was studied by *in vitro* autoradiography.

MATERIALS AND METHODS

Animals

Mouse model deficient in the low-density lipoprotein receptor and synthesizing only apolipoprotein B100 (LDLR$^{-/-}$ApoB$^{100/100}$, strain #003000, The Jackson Laboratory, Bar Harbor, ME) was utilized. The lipid profile of these mice closely resembles that of patients with familial hypercholesterolemia, and high-fat diet feeding leads to development of extensive atherosclerosis in the aorta[16,17,18]. The mice (n=10 for biodistribution+autoradiography and n=3 for PET/CT) were fed high-fat diet (TD88137, Harlan Laboratories, Madison, WI) for 4-5 months, starting at the age of 2-3 months. Healthy C57BL/6N mice (n=7 for biodistribution+autoradiography and n=4 for PET/CT) fed with regular chow were used as controls. All animal experiments were approved by the National Animal Experiment Board in Finland and the Regional State Administrative Agency for Southern Finland, and conducted in accordance with the European Union Directive.

Tracer Radiosynthesis

The method is described in the Online Resource 1.

In Vivo PET/CT

Three LDLR$^{-/-}$ApoB$^{100/100}$ mice and four C57BL/6N mice were imaged *in vivo* with PET/CT in order to study whole-body distribution and kinetics of ^{18}F-FEMPA. The imaging protocol and image analysis are described in the Online Resource 1.

Ex Vivo Biodistribution and Autoradiography

Ex vivo biodistribution was studied in ten LDLR$^{-/-}$ApoB$^{100/100}$ and seven C57BL/6N mice. Isoflurane-anesthetized mice were intravenously injected with 0.28±0.038 mCi (10±1.4 MBq) of ^{18}F-FEMPA and sacrificed 20 minutes afterwards. Blood was drawn via cardiac puncture and various tissues were excised and measured for radioactivity using gamma counter (Triathler 425-010, Hidex, Turku, Finland). The tissue radioactivity concentrations were expressed as standardized uptake values (SUV). For autoradiography, the thoracic aorta was embedded, frozen

and cut in sequential longitudinal 20 and 8-µm cryosections. The sections were apposed to an imaging plate (BAS TR2025, Fuji Photo Film Co., Ltd., Tokyo, Japan) for 4 hours followed by phosphoimager scanning (BAS-5000, Fuji Photo Film Co., Ltd., Tokyo, Japan).

In Vitro Autoradiography

The specificity of ^{18}F-FEMPA binding to TSPO in atherosclerotic plaques and vessel wall was studied *in vitro* by incubating tissue sections with the tracer in the presence and absence of an excess amount of unlabelled competitive binder (PK11195). Longitudinally cut 8-µm aortic sections from additional three LDLR$^{-/-}$ApoB$^{100/100}$ mice and two C57BL/6N mice, and tissue sections from carotid artery plaque endarterectomy samples from four patients with recent ischemic symptoms (three women, one man, age between 30 and 47) were utilized. The patient study protocol was accepted by the ethics committee of the Hospital District of Southwest Finland and the study was conducted according to the declaration of Helsinki.

Six sections from each sample were pre-incubated for 30 minutes in 50 mM Tris-HCl, followed by 30-minute incubation in Tris-HCl with 0.5 nM of ^{18}F-FEMPA (0.0011 mCi/mL [0.040 MBq/mL]), with or without 50 nM PK11195 (C0424, Sigma-Aldrich, St. Louis, MO). The sections were washed twice with ice-cold Tris-HCl, rinsed with distilled water, air-dried and apposed to an imaging plate for 2.5 hours followed by phosphoimager scanning.

Histology and Immunohistochemistry

The aortic 20-µm cryosections from the *ex vivo* autoradiography were stained with hematoxylin-eosin (H&E) for histology. Adjacent 8-µm sections were stained with anti-Mac-3 antibody for the detection of macrophages. The immunostaining of TSPO in cryosections was not feasible, and therefore, paraffin-embedded sections of aortic roots were prepared and stained with anti-TSPO or anti-Mac-3 antibody to show the co-localization. Murine aortic sections in the *in vitro* autoradiography were stained with H&E or with anti-Mac-3 antibody. The human carotid plaque sections were stained for macrophages with anti-CD68 antibody or vascular smooth muscle cells

(VSMCs) with anti-alpha-smooth muscle actin (α-SMA) antibody. The staining methods are described in the Online Resource 1.

Autoradiography Analyses

The autoradiographs were analyzed with Tina 2.1 software (Raytest Isotopemessgeräte, GmbH, Straubenhardt, Germany). The histology-based analysis was performed in the 20-µm sections in order to obtain optimal count intensities. Immunohistochemistry-based analysis was performed in the 8-µm sections, due the better quality of staining in the thin sections. After co-registration of outlines of stained section and the autoradiograph, count densities in the regions of interest (ROI) were analyzed based on histology. The ROIs in aortic longitudinal 20-µm sections were 1) non-calcified atherosclerotic plaque (plaque), 2) non-atherosclerotic vessel wall distant from plaques (wall), 3) adventitia including perivascular adipose tissue (adventitia) and 4) calcified area in the plaque (calcification). The average numbers of analyzed ROIs per mouse were 49 for plaque, 30 for wall, 32 for adventitia and 4 for calcification. The radioactivity uptake was expressed as count density (photo-stimulated luminescence per square millimetre, PSL/mm^2) with the background subtracted, normalized for the injected radioactivity dose/ mouse weight and the radioactivity decay during exposure (See Online Resource 1). An average count density for each ROI was calculated for each mouse.

Because of the heterogeneous distribution of ^{18}F-FEMPA within the plaques, a more detailed analysis was performed. In order to study the co-localization of tracer uptake and macrophages, the tracer distribution within the plaque areas was analyzed by autoradiography of 8-µm sections later stained with anti-Mac-3 antibody. ROIs were defined into non-atherosclerotic vessel wall and areas of plaques representing different macrophage densities, graded as 0) no macrophages, 1) low (occasional macrophages), 2) intermediate (scattered groups of macrophages) or 3) high (confluent areas of macrophages). Average background-subtracted and normalized count densities were calculated.

In the *in vitro* autoradiographs of human carotid plaque sections, ROIs were defined in areas positive for CD68 or α-SMA, and in murine aortic sections, ROIs were defined in plaque and wall. Average background-subtracted count densities were calculated.

Statistical Analysis

All results are expressed as mean ± standard deviation. Statistical analysis was performed with IBM SPSS Statistics 21 (IBM Corp., Armonk, NY). Independent-sample *t*-test was used for calculating differences between atherosclerotic and control mice. Paired *t*-test was used for comparing uptake between different tissues of atherosclerotic mice, and for comparisons of ^{18}F-FEMPA binding in different regions in the *in vitro* autoradiography. Analysis of variance with Tukey HSD correction was used in multiple comparisons. The *P* values lower than 0.05 were regarded as statistically significant.

RESULTS

Characterization of Atherosclerotic Lesions in Mice

The aortas of healthy controls did not show atherosclerosis, whereas LDLR$^{-/-}$ApoB$^{100/100}$ mice showed large aortic fibroatheroma-type lesions with occasional small calcifications. Based on Mac-3 staining, all plaques showed macrophage infiltration that varied from occasional to large confluent areas of macrophages. The vessel wall outside of the plaques was apparently normal.

The immunohistochemistry of LDLR$^{-/-}$ApoB$^{100/100}$ aortic root sections revealed that TSPO and Mac-3 co-localized in the plaque intima (Figure 1). Positive TSPO staining was also observed in VSMCs, endothelial cells and brown adipose tissue surrounding the vessel. Some cells positive for TSPO were also present in plaque areas that were Mac-3 negative. In healthy mice, TSPO positivity was observed throughout the vessel wall, whereas Mac-3 staining was negative. The control stainings without primary antibody were consistently negative.

In Vivo PET/CT Imaging

Dynamic PET/CT showed rapid clearance of ^{18}F-FEMPA from the blood. The remaining radioactivity concentration (SUV) in the blood pool was 1.5±0.11 at 40 minutes post-injection. A rapid accumulation of ^{18}F-FEMPA in the lungs, peaking at 1-2 minutes post-injection, was observed. High radioactivity concentration was also observed in the adrenal glands, kidneys and liver (Figure 2a). Uptake of ^{18}F-FEMPA was lower in atherosclerotic than control mice in in the adrenal gland (2.5±0.11 vs. 3.9±0.72, P=.021) at 40 minutes post-injection, otherwise no significant differences in the *in vivo* SUVs were observed between the strains (Figure 2b-c). There was visually detectable ^{18}F-FEMPA uptake that co-localized with the aortic arch (Figure 2d), but no significant difference between atherosclerotic and control mice (0.83±0.10 vs. 0.94±0.14, P=.32). Since the radioactivity concentrations in most tissues reached a plateau already after 15 to 20 minutes post-injection, the 20-minute time point was selected for *ex vivo* measurements.

Ex Vivo Biodistribution

The uptake of ^{18}F-FEMPA (SUV) was significantly higher in atherosclerotic aortas (2.4±0.61) than in the blood of the same mice (0.49±0.89, *P*<.001) or the aortas of healthy control mice (1.9±0.23, *P*=.028). The radioactivity concentration in the plasma was also significantly higher in atherosclerotic mice when compared to healthy controls (0.37±0.083 vs. 0.21±0.10, *P*=.0018), whereas the liver uptake was lower (2.0±0.42 vs. 2.7±0.88, *P*=.028). The highest radioactivity concentrations were observed in adrenal glands and lungs. Detailed results of the ^{18}F-FEMPA *ex vivo* biodistribution are shown in Table 1.

Ex Vivo Autoradiography

The autoradiography showed ^{18}F-FEMPA uptake in the vessel wall throughout the aorta both in atherosclerotic and control mice (Figure 3). The average uptake in atherosclerotic plaques was lower than in the non-atherosclerotic vessel wall of LDLR$^{-/-}$ApoB$^{100/100}$ mice (count densities 270±85 vs. 570±89 PSL/mm^2, *P*<.001). There was no difference in tracer uptake in the non-atherosclerotic vessel wall between atherosclerotic mice and controls (570±89 vs. 680±270 PSL/mm^2, *P*=.29). Uptake in the adventitia was 200±98 PSL/mm^2, and the uptake in calcifications 150±64 PSL/mm^2.

The detailed autoradiography analysis of 8-µm sections revealed that the highest ^{18}F-FEMPA uptake in the plaques co-localized with the macrophage-rich areas in the same sections (Figure 3). The count densities were 40±13, 67±22, 140±30 and 190±54 PSL/mm^2 in the areas of no macrophages, low, intermediate and high macrophage density, respectively. The uptake was significantly higher in high macrophage density areas than areas with only few macrophages (*P*<.001). The uptake in the most inflamed plaque areas was comparable with the uptake in the non-atherosclerotic vessel wall in the same sections (230±78 PSL/mm^2, *P*=.40).

In Vitro Autoradiography

The human carotid endarterectomy samples showed CD68 positive areas in all except one sample, which contained fibrotic and calcified tissue without visually detectable macrophage infiltration. ^{18}F-FEMPA binding was observed predominantly in CD68-positive areas (760± 520 PSL/mm^2), whereas in the α-SMA-positive areas tracer accumulation tended to be lower (230 ±

220 PSL/mm^2, *P*=.091) (Figure 4, Table 2). In the presence of 100x molar excess of PK11195, the count densities were lowered by 75 ± 13 % in the CD68 positive areas (160 ± 65 PSL/mm^2, *P*=.15) and by 79 ± 12 % in the α-SMA-positive areas (38 ± 21 PSL/mm^2, *P*=.14). In the mouse aortic sections, ^{18}F-FEMPA showed accumulation *in vitro* both in the plaques and the vessel wall, similarly as in *ex vivo* autoradiography. The blocking with unlabelled PK11195 lowered the count densities by 79 ± 3.5 % in the plaques (*P*=.0058), 84 ± 0.060 % in the vessel wall of atherosclerotic mice (*P*<.001), and by 79 ± 1.1 % in the vessel wall of control mice (*P* =.036).

DISCUSSION

The aim of this study was to evaluate the potential of the new TSPO ligand, ^{18}F-FEMPA, in the detection of atherosclerotic plaque inflammation. Our results demonstrate that ^{18}F-FEMPA is rapidly cleared from blood and shows uptake in mouse aorta. Accumulation of ^{18}F-FEMPA in atherosclerotic plaques was related to the amount of macrophages. However, in this model, uptake in atherosclerotic lesions did not exceed that in the healthy vessel wall.

Our ^{18}F-FEMPA biodistribution results in mice are in accordance with previous findings of TSPO distribution[19,20]. Accumulation of ^{18}F-FEMPA in the atherosclerotic aorta was high compared to blood, consistent with previous results with ^{11}C-PK11195 in the same mouse model[21]. The observed high uptake in the lung is similar as observed with ^{11}C-PK11195, and might limit the use of this tracer in the imaging of coronary arteries or aorta adjacent to the lung[22,23].

The analysis of ^{18}F-FEMPA distribution in the aortic *ex vivo* autoradiography showed accumulation of tracer in atherosclerotic plaques with abundant macrophage infiltration. Moreover, intra-plaque ^{18}F-FEMPA uptake co-localized with macrophage-rich areas. We also showed accumulation of ^{18}F-FEMPA in areas with CD68-positive macrophages in human carotid plaque sections *in vitro*. These observations are consistent with high TSPO expression by macrophages[10] and previous studies showing *in vitro* binding of ^{3}H-PK11195 in human atherosclerotic plaques containing CD68-positive macrophages[3,13,14]. However, the ^{18}F-FEMPA uptake, even in plaques with high macrophage density, was not higher than the uptake in the non-atherosclerotic vessel wall. This uptake ratio between atherosclerotic plaque and non-atherosclerotic vessel wall appears comparable to that of ^{11}C-PK11195[21], suggesting that the utility of ^{18}F-FEMPA for visualization of atherosclerotic lesions may be limited by low signal-to-noise ratio. However, like ^{11}C-PK11195, ^{18}F-FEMPA might be suitable for detection of vascular inflammation in conditions associated with more intense inflammation, such as large vessel vasculitis[22,23].

The uptake of ^{18}F-FEMPA in normal aortic wall can be explained by the binding of the tracer in TSPO-positive endothelial cells and VSMCs. However, the expression of TSPO in macrophages has been reported to be 20-fold higher compared to VSMCs[3]. Despite the generally similar expression pattern of TSPO is in rodents and humans, some differences between species have

been observed. For example, the expression is high in the VSMCs in rats, but not in humans[24,25]. In the current study, the *in vitro* autoradiography of human carotid artery plaque showed 3.2-fold higher ^{18}F-FEMPA binding in the CD68-positive areas as compared to α-SMA-positive areas, and previous autoradiographic studies of human carotid arteries have shown no accumulation of ^{3}H-PK11195 in the VSMCs[13,14]. The feasibility of ^{18}F-FEMPA in the imaging of vascular atherosclerotic plaque inflammation in humans remains to be tested.

The results of *ex vivo* gamma counting showed higher SUV of ^{18}F-FEMPA uptake in the aortas of atherosclerotic mice than healthy controls. This appears discrepant with analysis of tracer distribution by autoradiography, which showed less uptake in atherosclerotic plaques than in the normal vessel wall, and no detectable differences in the healthy vessel wall uptakes between the atherosclerotic and control animals. This probably reflects the different nature of these methods: radioactivity is quantified in the whole tissue sample by gamma counting, whereas autoradiography is focused on specified regions in tissue sections and does not cover the whole sample. There were also some discrepancies between the measured *in vivo* and *ex vivo* tracer distribution, such as lower activity in the adrenal glands measured *in vivo* than *ex vivo*. These may be explained by partial volume effect. Higher blood pool radioactivity concentration in the *in vivo* data could be explained by high radioactivity in the vessel wall of vena cava causing spillover to the blood ROI. On the contrary, the *in vivo* uptake in the aorta is lower than the uptake measured *ex vivo*, since it is not possible to define the ROI only in the aortic wall and the blood pool radioactivity causes interference.

Limitations

The *in vivo* PET/CT imaging did not show differences in aortic ^{18}F-FEMPA uptake between the atherosclerotic and healthy animals, although the difference was significant *ex vivo*. The reason for this might be the low spatial resolution of PET and the observed ^{18}F-FEMPA uptake in normal vessel wall. Furthermore, there may be species differences in TSPO expression in different tissues. Therefore, the *in vivo* imaging results of vascular tracer uptake need to be interpreted with caution.

New knowledge gained

Novel TSPO-targeting PET tracer ^{18}F-FEMPA shows uptake in the mouse aorta and its uptake in atherosclerotic plaques correlates with the amount of macrophages. However, plaque uptake does not exceed that in the normal vessel wall.

CONCLUSION

Our results demonstrate that ^{18}F-FEMPA shows uptake in mouse aorta. The association of ^{18}F-FEMPA uptake with macrophage-rich plaque areas suggests that this novel TSPO tracer may have potential for the assessment of vascular inflammation. However, uptake in plaques did not exceed that in the normal vessel wall. Since ^{18}F-FEMPA has been used in human studies already[15], *in vivo* imaging of vascular inflammation in atherosclerosis and other conditions, such as vasculitis, associated with more intense vascular inflammation could potentially be tested in clinical trials.

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Conflict of interest disclosure

AT, LL, TH and SV were employed by Bayer Pharma AG, Berlin, Germany at the time of the study. Other authors declare no conflict of interest. The compound ^{18}F-FEMPA is now part of the portfolio of the Piramal Imaging GmbH.

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FIGURE LEGENDS

Fig.1 Representative immunostainings. (a) Mac-3 stained section of atherosclerotic LDLR$^{-/-}$ ApoB$^{100/100}$ aortic root and (b) TSPO staining of adjacent section. Positive staining (brown color) is observed with both antibodies in the intima (red arrows) and in the media (black arrows). TSPO staining is present also in some Mac-3-negative areas (green arrows). (c) Mac-3 staining is negative in healthy C57BL/6N mouse aortic root section, whereas (d) positive TSPO staining is present in the vascular smooth muscle cells and endothelium (arrows).

Fig.2 *In vivo* PET/CT imaging with ^{18}F-FEMPA. (a) Coronal PET/CT image represents radioactivity distribution at 40 minutes post-injection. (b) Time-activity curves of selected tissues represent mean of three atherosclerotic mice. (c) Mean time-activity curves of four healthy mice. (d) Detailed view of atherosclerotic mouse thorax. A=aorta, L=lungs, Li=liver, M=myocardium. ^{18}F-FEMPA uptake is seen in liver, lungs and myocardium, as well as co-localizing with the aortic arch (arrows in d).

Fig.3 The autoradiography and histology of atherosclerotic mouse aorta. (a) Longitudinally cut, hematoxylin and eosin stained section of atherosclerotic mouse aorta. AA=ascending aorta; DA=descending aorta; L=lumen; B=brachiocephalic artery, C=carotid artery and S=subclavian artery. The plaques are circled. (b) Mac-3-stained consecutive section of the same aorta. Four areas of plaque are annotated with a number referring to its grade of macrophage density (0-3). Positive staining is seen as brown color. Grade 0 (no macrophages), grade 1 (occasional macrophages), grade 2 (groups of macrophages), grade 3 (confluent area of macrophages). W=normal vessel wall. (c) *Ex vivo* autoradiograph of aortic section shows strong ^{18}F-FEMPA uptake (red) in some atherosclerotic plaques as well as the non-atherosclerotic vessel wall. (d) Quantitative results of ^{18}F-FEMPA uptake. * Significant difference from uptake in vessel wall, P=0.0023, ** Significant difference from uptake in vessel wall, P<0.001. # Significant difference

from uptake in grade 0 areas, *P*=0.0017, ## Significant difference from uptake in grade 0 areas, *P*<0.001.

Fig.4 *In vitro* binding of ^{18}F-FEMPA into human atherosclerotic plaque. (a) ^{18}F-FEMPA autoradiography shows high tracer binding in red. (b)^{18}F-FEMPA autoradiography with 100x molar excess of PK11195 shows significantly less tracer binding. (c) Positive staining for macrophages (CD68) is observed in the same areas as high tracer binding. (d) Staining for smooth muscle cells (α-SMA).

TABLES

Table 1. *Ex vivo* biodistribution of ^{18}F-FEMPA radioactivity at 20 minutes post-injection in atherosclerotic LDLR$^{-/-}$ApoB$^{100/100}$ and healthy C57BL/6N mice.

	LDLR$^{-/-}$ApoB$^{100/100}$ n=10[a]	C57BL/6N n=7[b]	P value
Adrenal gland	26±24	57±50	
Aorta	2.4±0.61	1.9±0.23	.028
Blood	0.49±0.089	0.53±0.091	
Kidney	14±2.7	13±2.9	
Liver	2.0±0.42	2.7±0.88	.028
Lungs	27±19	42±57	
Lymph node	2.4±0.54	2.9±2.1	
Muscle	0.35±0.15	0.32±0.12	
Myocardium	6.4±1.9	6.7±1.8	
Plasma	0.37±0.083	0.21±0.10	.0018
Urine	11±7.9	1.2±0.61	
WAT	0.085±0.034	0.092±0.022	

Results are expressed as standardized uptake values (mean ± standard deviation). Significant *P* values are reported.

[a] lung *n*=9, urine and lymph node *n*=8

[b] urine *n*=3

Table 2. The results of ^{18}F-FEMPA *in vitro* autoradiography of human carotid endarterectomy plaque sections.

Patient	CD68	α-SMA	CD68 blocked	α-SMA blocked
A	330	83	130	32
B	1300	550	230	67
C	610	180	110	37
D	NA	110	NA	16

Results are expressed as photo-stimulated luminescence per square millimeter (PSL/mm^2). CD68: areas positive for macrophages, α-SMA: areas positive for smooth muscle cells. One of the plaques did not contain CD68 positive areas. The blocked results are obtained from incubation with 100× molar excess of unlabeled PK11195. NA: not applicable.