ELECTRONIC SUPPLEMENTARY MATERIAL/ ONLINE RESOURCE 1

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

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SUPPLEMENTAL MATERIALS AND METHODS

Tracer Radiosynthesis

^{18}F-FEMPA was prepared according to the previous method[1] with slight modifications. No-carrier-added aqueous ^{18}F-fluoride ion was produced by irradiation of a niobium-bodied oxygen-18 enriched water target (Rotem, >98%) with 17 MeV protons and a beam current of 40 µA (cyclotron from Efremov Institute of Elektrophysical Apparatuses, St Petersburg, Russia). ^{18}F-FEMPA was synthesized by nucleophilic fluorination of FEMPA-mesylate precursor supplied by Bayer Schering Pharma/Piramal Imaging SA (Matran, Switzerland) for this project. The labeled product was separated by preparative HPLC followed by a wash- and formulation-procedure using a solid phase extraction C18 cartridge. The sterile end product was formulated in phosphate buffered saline (pH 7.4) containing 10% ethanol. The content of 18F+19F-FEMPA + sum of chemical impurities in the end product was <10 µg/mL and the radiochemical purity of ^{18}F-FEMPA was higher than 99%. The specific (radio)activity was >91GBq/ µmol.

In Vivo PET/CT Imaging

Under isoflurane anesthesia, following a CT for attenuation correction, a 40-minute dynamic PET was performed starting at the same time as intravenous (i.v.) ^{18}F-FEMPA injection (9.5 ± 0.35 MBq). Immediately after PET, a contrast agent (100-200 µL; eXia 160XL, Binitio Biomedical, Ottawa, Canada) was i.v. injected and a high-resolution CT was performed for anatomical reference. CT acquisition consisted of 161 projections with the exposure time of 1300 ms, x-ray voltage of 80 kVp, and anode current of 500 µA for a 220° rotation.

PET data acquired in a list mode were iteratively reconstructed with a 2-dimensional ordered-subsets expectation maximization algorithm into 30 × 10 s, 15 × 60 s and 2 × 600 s frames. A reconstructed image had 128 × 128 × 159 matrix size with a pixel size of 0.776 × 0.776 × 0.796 mm. CT images were reconstructed with a filtered back-projection algorithm (pixel size 0.109 × 0.109 × 0.109 mm).

After a carefully performed PET and CT co-registration, the images were analyzed with Carimas 2.9 software (Turku PET Centre; http://www.turkupetcentre.fi/carimas)[2]. Three-dimensional volumes of interest (VOIs) were defined into various tissues and their time-activity curves were extracted. VOI for blood circulation was located in vena cava inferior at the level of kidneys; myocardial VOI was located in the apex of the heart. The radioactivity concentrations in various tissues were expressed as standardized uptake values (SUV).

Immunohistochemistry

For macrophage staining (CD68), the frozen sections of human carotid artery plaques were thawed and fixed in acetone. Endogeneous peroxidase was blocked with 1 % hydrogen peroxidase, followed by incubation with horse serum (Vectastain ABC-kit PK-4002, Vector Laboratories Inc., Burlingame, CA). Anti-CD68 antibody (Monoclonal mouse anti-human CD68, clone PG-MI, 1:200, DakoCytomation, Glostrup, Denmark) was incubated overnight at 4°C. Biotinylated anti-mouse IgG (Vectastain-kit) was incubated for 30 minutes followed by ABC-reagent (Vectastain-kit) and 3,3' diaminobenzidine tetrahydrochloride hydrate (DAB). The sections were counterstained with hemaytoxylin. Smooth muscle cells were stained with similar protocol, except that the sections were fixed with 4% formaldehyde. The primary antibody was anti-alfa-smooth muscle actin (α-SMA, 1:15000, clone 1A4, Sigma-Aldrich, St. Louis, MO).

Murine aortic 8-μm cryosections were stored at –70°C, thawed and fixed for 10 min in 4% formaldehyde. Mac-3 antigen was uncovered by boiling in hot 10 mM citrate buffer (pH 6.0) for 20 minutes, followed by 1-hour incubation with primary antibody (rat anti-mouse Mac-3, clone M3/84, 1:5000, BD Pharmingen, Franklin Lakes, NJ). Endogenous peroxidase was blocked with 1% hydrogen peroxide, and the sections were incubated for 30 minutes with polyclonal rabbit anti-rat antibody (DakoCytomation E0468, 1:200) and for 30 min with tertiary EnVision+ System- HRP-labelled goat anti-rabbit antibody (DakoCytomation EnVision anti-rabbit K4003). Detection was performed with DAB and substrate (DakoCytomation K3468) Mayer’s haematoxylin was used for counterstaining.

Aortic roots of additional three LDLR$^{-/-}$ApoB$^{100/100}$ and two C57BL/6N mice were prepared and stained with anti-TSPO or anti-Mac-3 antibody. For Mac-3 immunohistochemical staining, the de-paraffinized and rehydrated sections were first blocked with 3% BSA followed by 1 h incubation with the primary antibody (rat anti-mouse Mac-3, clone M3/84, 1:500, BD Pharmingen, Franklin Lakes, NJ). Endogenous peroxidase was blocked with 3% H_2O_2 in Tris-HCl for 10 min. The following steps in staining were similar as described above. For TSPO immunohistochemical staining, the formalin-fixed, paraffin-embedded sections of aortic root were first de-paraffinized and rehydrated. After a pre-incubation in 10 mM citrate buffer and blocking with 3% bovine serum albumin (BSA), sections were incubated with the primary antibody (NBP1-95674, 1:10000, Novus Biologicals) for 1 h. Endogenous peroxidase was blocked with 3% H_2O_2 in Tris-HCl for 10 min followed by 30 min incubation with the horseradish peroxidase conjugated secondary antibody (Dako EnVision+System HRP goat anti-rabbit K4003). Detection was performed as described above. Negative control stainings were performed without the primary antibody.

Autoradiography normalization method

The radioactivity uptake in autoradiography was expressed as count density (photo-stimulated luminescence per square millimetre, PSL/mm^2) and the background counts were subtracted. In order to minimize the effects of variations in the injected dose and autoradiography exposure timing, the obtained count densities were normalized for the injected radioactivity dose/ mouse weight (mCi/g) and the proportion of radioactivity which decayed during the autoradiography exposure (decay percentage). The decay percentage was calculated by determining the percentage of radioactivity remaining in the tissue at the start of autoradiography exposure and subtracting the percentage of radioactivity remaining in the tissue at the end of the exposure. The PSL/mm^2 values were normalized to the calculated median value of mCi/g and decay percentage. The normalized values were obtained by the calculation: PSL/mm^2 * (mCi/g$_{median}$ / mCi/g$_{mouse}$) * (decay percentage$_{median}$ / decay percentage$_{mouse}$).

SUPPLEMENTAL REFERENCES

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