Currently, the likelihood of carotid atherosclerotic plaque rupture is assessed by the degree of vessel stenosis. High macrophage content is a consistent finding in ruptured plaques and represents a potentially superior measure of plaque instability. The translocator protein/peripheral benzodiazepine receptor (TSPO/PBR) is highly expressed in macrophages (Libby, 2002) and can be imaged by positron emission tomography (PET). Thus, PET-radiolabelled TSPO ligands have the potential to accurately quantify plaque inflammation and assess potential plaque instability by non-invasive means. Our aim was to evaluate two radioligands, PK11195 and DAA/FEDAA in human carotid atherosclerotic plaque.

Human tissues were obtained with ethical approval and patient consent. Human vascular smooth muscle cells (VSMC) were obtained from carotid surgical tissue and monocytes from peripheral blood. Binding analysis was performed with 1 to 24nM $[^3H]PK11195$, 3.15TBq/mmol, 37MBq/mL and non-specific binding was determined with 2μM unlabelled PK11195. Binding characteristics were calculated from Scatchard plots. Protein was determined by the Biuret assay. Macrophage-specific TSPO expression was determined in six carotid atherosclerotic plaques obtained from surgical specimens. Fresh frozen sections of 30 μm were used in receptor autoradiography with 5nM $[^3H]PK11195$, 5nM $[^11C]PK11195$ (100 to 200TBq/mmol) and 0.25nM $[^3H]DAA1106$ (2.81TBq/mmol, 74MBq/mL) and immunohistochemistry for macrophages (mouse anti-human CD68 monoclonal antibody). Signal expression for CD68 and specific $[^3H]PK11195$, $[^11C]PK11195$ and $[^3H]DAA$ binding was captured by microscopy and digital images of sections were analysed using MatLab software. Statistical correlation was determined using Spearman’s $\rho$ in SPSS.

$[^3H]PK11195$ bound to TSPO in cultured human VSMCs and monocytes with similar affinities (VSCMs Kd = 5.5+/−0.6nM; monocytes Kd = 3.7+/−1.1nM). In contrast, receptor density was much higher in monocyte cultures (Bmax = 8.837+/−0.8pmol/mg) compared with VSMCs (Bmax = 425.8+/−16.3fmol/mg). The specific binding of PK11195 and DAA were compared in six atherosclerotic plaques in vitro. Significant correlation in specific binding was found between $[^3H]PK11195$, $[^11C]PK11195$ and $[^3H]DAA1106$ (P<0.05). Although areas of plaque which were positive for CD68 presence demonstrated co-incidence with both PK11195 and DAA expression, this was only statistically significant for DAA ($R^2$=0.94, P=0.0048).

The TSPO/PBR demonstrates considerable difference in expression between monocytes and VSMCs and thus, has the potential to quantify macrophages in carotid atherosclerotic plaques. PK11195 has previously been shown to bind in macrophage-rich regions in human carotid plaque in vitro (Fujimura et al., 2008). We have shown that the DAA has the same cellular distribution as PK11195 in the plaque. The superior binding affinity of DAA for TSPO (Maeda et al., 2004) makes this ligand an excellent candidate for further evaluation for in vivo PET-based quantification of the inflammation associated with atherosclerosis.

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