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A microPET study of the regional distribution of [^{11}C]-PK11195 binding following temporary focal cerebral ischemia in the rat. Correlation with post mortem mapping of microglia activation

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ABSTRACT

Background: Post-stroke microglial activation (MA) may have both neurotoxic and pro-repair effects, particularly in the salvaged penumbra. Mapping MA *in vivo* is therefore an important goal. ^{11}C-PK11195, a ligand for the 18 kDa translocator protein, is the reference radioligand for MA imaging, but a correlation between the regional distributions of *in vivo* tracer binding and *post mortem* MA after stroke, as assessed with PET and immunohistochemistry, respectively, has not been demonstrated so far. Here we performed ^{11}C-PK11195 microPET in a rat model previously shown to induce extensive cortical MA, and determined the correlation between ^{11}C-PK11195 and immunostaining with the CD11 antibody OX42, so as to verify the presence of activated microglia, in a template of PET-resolution size regions-of-interest (ROIs) spanning the whole affected hemisphere.

Methods: Adult spontaneously hypertensive rats underwent 45 min distal middle cerebral artery occlusion and ^{11}C-PK11195 PET at Days 2 and 14 after stroke according to a longitudinal design. Following perfusion-fixation at Day 14, brains were removed and coronally cut for OX42 staining. ^{11}C-PK11195 binding potential (BP_{ND}) parametric maps were generated, and in each rat both BP_{ND} and OX42 (intensity × extent score) were obtained in the same set of 44 ROIs extracted from a cytoarchitectonic atlas to cover the whole hemisphere. Correlations were computed across the 44 ROIs both within and across subjects.

Results: Significant BP_{ND} increases were observed in both the infarct and surrounding areas in all rats at day 14; less strong but still significant increases were present at day 2. There were highly significant (all $p<0.001$) positive correlations, both within- and across-subjects, between day 14 BP_{ND} values and OX42 scores.

Conclusions: The correlation between Day 14 ^{11}C-PK11195 and OX42 across the affected hemisphere from the same brain regions and animals further supports the validity of ^{11}C-PK11195 as an *in vivo* imaging marker of MA following stroke. The finding of statistically significant increases in ^{11}C-PK11195 as early as 48 h after stroke is novel. These results have implications for mapping MA after stroke, with potential therapeutic applications.

Introduction

After a stroke, microglial activation (MA) may be neurotoxic but may also facilitate neuroplasticity in the peri-infarct zone (del Zoppo et al., 2001). MA is therefore a potential therapeutic target, and as such an important pathophysiological process to investigate after stroke. Using selective immunohistochemistry (IHC) markers such as OX42, an antibody which binds to CD11, a protein selectively expressed by activated microglia, MA can be documented *post mortem* after MCA occlusion (MCAo) in rodents in both the infarct and the peri-infarct areas (Hughes et al., 2010; Lehrmann et al., 1997; Myers et al., 1991; Schroeter et al., 2009). MA can also be studied using radioligands specific to the translocator protein (TSPO; previously known as the peripheral benzodiazepine receptor, PBR), which is also massively expressed by activated microglia (Banati, 2002). Previous work in an axotomy model has demonstrated the association between *in vitro* CD11 and TSPO binding (Pedersen et al.,

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2006). Using the reference ligand ^{3}H-[R]-PK11195 (Chauveau et al., 2008), which co-localizes to resident activated microglia as shown by emulsion autoradiographic (ARG) studies (Banati et al., 1997), seminal rodent MCAo studies documented several-fold increases in TSPO binding relative to normal brain (Benavides et al., 1990; Dubois et al., 1988; Myers et al., 1991).

PK11195 was subsequently labeled with ^{11}C for PET applications (Chauveau et al., 2008). Interestingly, although PET studies using ^{11}C- PK11195 have consistently reported low specific binding in the normal brain across species, ^{3}H-PK11195 binding is substantial *in vitro* (*e.g.* Bmax range in gray matter structures ~200–400 pmol/g in the mouse and 200–600 pmol/g in the pig) (Cumming et al., 2006; Pedersen et al., 2006), suggesting some state difference in the TSPO between the *in vivo* and *in vitro* conditions (Cumming et al., 2006). Increased ^{11}C-PK11195 binding has been consistently reported after stroke in both non-human primates and man (Gerhard et al., 2000; Gerhard et al., 2005; Price et al., 2006; Ramsay et al., 1992; Sette et al., 1993; Thiel et al., 2010), and similar findings have been recently reported using microPET in rodents (Fukumoto et al., 2011; Rojas et al., 2007; Schroeter et al., 2009).

In order to fully exploit the potential of ^{11}C-PK11195 as an *in vivo* marker of MA in stroke, it is important to ensure that its binding as assessed with PET does reflect MA, as expected from the above studies linking CD11 to TSPO binding (Pedersen et al., 2006). *In vitro* or *ex vivo* studies using ^{3}H-PK11195 in rodent models of cerebral ischemia have reported co-localization of the radiotracer with resident microglia and macrophages (Myers et al., 1991; Stephenson et al., 1995) as well as a topographical correlation with IHC markers of MA but not GFAP (Myers et al., 1991). In two recent PET studies, ^{11}C-PK11195 uptake showed overall topographical congruence with *post mortem* OX 42 at the microscopic level, but a direct correlation using the same regions-of-interest (ROIs) in the same subjects was not reported (Fukumoto et al., 2011; Rojas et al., 2007), while another study found congruence in the peri-infarct areas but not in the core (Schroeter et al., 2009). Thus, no study so far has directly investigated the relationship between the regional distribution of ^{11}C-PK11195 binding assessed with PET, and that of MA assessed *post mortem* using IHC, in the same ROIs and subjects and at the spatial resolution of PET, after experimental stroke.

Using OX42, we recently documented the occurrence of extensive MA two weeks after 45 min temporary MCAo (tMCAo) in spontaneously hypertensive rats (SHRs), involving both the infarct and non-infarcted areas acutely affected by penumbral hypoperfusion (Hughes et al., 2010). Here we report the regional distribution of ^{11}C-PK11195 assessed in the same series of SHRs scanned at Day 14 post-MCAo, an optimal time to detect MA based on ARG studies (Benavides et al., 1990; Cremer et al., 1992), and investigate the correlation between ^{11}C-PK11195 binding and *post mortem* OX42 throughout the whole MCA territory using ROIs of size consistent with PET resolution. To address this aim, we employed our previously validated semi-quantitative method for assessing IHC within a template of ROIs spanning the MCA territory, designed to be comprehensive, allow correlation with PET data, and at the same time avoid operator bias as far as possible (Hughes et al., 2010). In addition, PET was also performed in the same subjects at day 2 to address discrepancies in the literature regarding ^{11}C-PK11195 findings at this very early time point (Fukumoto et al., 2011; Myers et al., 1991; Ramsay et al., 1992; Rojas et al., 2007; Sette et al., 1993).

Materials and methods

To comply with UK Home Office regulations, this study was designed so the number of animals used be as small as possible yet adequate for scientifically robust results. Thus, the planned ^{11}C-PK11195-OX42 relationship was to be tested not by group comparison but within animals. Accordingly, a sample of six rats was judged adequate. All animals were treated in accordance with the UK Animals Scientific Procedures Act 1986 and Cambridge University Ethical Review Panel.

According to a longitudinal design, each rat was to undergo 45 min tMCAo at day 0, ^{11}C-PK11195 PET at days 2 and 14, and perfusion-fixation for brain collection immediately following completion of the day 14 PET scan

Detailed account of the generic anesthetic, surgical, PET and post-mortem procedures have been published previously (Hughes et al., 2010; Takasawa et al., 2007), so only a summary will be given here.

Experimental procedures

General

Male ~3-month old SHRs weighting 280–330 g (Charles River, UK) were anesthetized with N_2O/O_2 (0.7/0.3 L/min), augmented with isoflurane (4% for induction and 2% thereafter). Body temperature was maintained at 37.0 ± 0.5 °C using a heated pad, and oxygen saturation was monitored using an oximeter. The main physiological variables, including arterial PO_2, PCO_2, pH, HCO_3 and O_2 saturation were within normal ranges. Although not measured in these rats in order to avoid the added procedural complications including blood loss related to femoral artery cannulation, blood pressure is known to be already significantly elevated by 3 months of age in SHRs (Amenta et al., 2010; Leoni et al., 2011).

tMCAo

The distal clip MCAo model (Buchan et al., 1992) was used as implemented in our laboratory (Takasawa et al., 2007). A loose ligature was placed around the right common carotid artery (CCA). The right MCA was exposed via a 2-mm burr hole and occluded with a micro-aneurysm clip (No 1, Johnson & Johnson), and the right CCA permanently occluded. After 45 min the clip was removed and wound closed. Restoration of blood flow was verified visually.

^{11}C-PK11195 PET

For both the day 2 (*i.e.*, 48 h after tMCAo) and day 14 PET studies, animals were reanesthetized as above, a venous cannula inserted in the tail vein and the subject positioned in a purpose-built plastic frame incorporating ear bars and a bite bar at the center of a microPET P4 scanner (Concorde Microsystems, Knoxville, TN). High specific activity (mean 181 MBq/nmol) ^{11}C-PK11195 was injected i.v. as a 1 ml bolus. The mass of administered PK11195 was purposely made as similar as possible in all rats (2.1 ± 2.4 nmol/kg) by adjusting the injected activity (203 ± 84 MBq). Under 1–1.5% isoflurane, PET data was acquired in list mode (350–650 keV energy window, 6 ns timing window) for 90 min and binned into the following time frames: 10 × 0.5 + 5 × 1 + 15 × 2 + 10 × 5 min. Prior to scanning an 11 min coincidence mode ^{68}Ge transmission scan was acquired for attenuation correction.

Image processing

The images were reconstructed into 0.5 × 0.5 × 0.5 mm voxels using 3D filtered backprojection with a Hann window cut-off at the Nyquist frequency (final resolution ~2.3 mm FWHM isotropic). Corrections were applied for background, randoms, dead time, normalization, attenuation, decay and sensitivity.

For each scan, an early (*i.e.*, perfusion-weighted) PET image was obtained by summing the 0–5 min frames, and was co-registered to a T2-weighted MRI template obtained in a healthy SHR of same gender and similar age and weight as used in this study, using MPI Tool software (Max Planck Institute, Cologne, Germany). This allowed all the PET images to be resliced into a standard 3D space using SPM2 (www.fil.ion.ucl.ac.uk/spm). Parametric images of regional ^{11}C-PK11195 non-displaceable binding potential (BP_{ND}) were produced