

Intrinsic Activated Microglia Map to the Peri-infarct Zone in the Subacute Phase of Ischemic Stroke

Christopher J.S. Price, MRCP; Dechao Wang, PhD; David K. Menon, PhD; Joe V. Guadagno, MRCP; Marcel Cleij, PhD; Tim Fryer, PhD; Franklin Aigbirio, PhD; Jean-Claude Baron, MD; Elizabeth A. Warburton, DM

Background and Purpose—Microglial activation is an important component of the neuroinflammatory response to ischemic stroke. Experimental studies have outlined such patterns temporally and spatially. In vivo studies in stroke patients have relied on positron emission tomography and (R)-PK11195, a ligand that binds peripheral benzodiazepine binding sites. In this study we sought to establish temporal and spatial patterns of microglial activation in ischemic stroke with particular emphasis on a defined peri-infarct zone.

Methods—Using this technique, we studied carotid territory ischemic stroke patients in 3 time windows up to 30 days after ictus. Controls were studied in a single session. [^{11}C](R)-PK11195 injection was followed by 3-dimensional acquisition over 60 minutes. Cerebral blood volume (CBV) was measured afterward with the use of standard C^{15}O paradigms. Analysis employed the reference tissue model in which ipsilateral cerebellum was used to generate parametric binding potential maps corrected for CBV. Data were coregistered to T1-based MRI. Using control data to identify 99% confidence limits, a region of interest analysis was applied to identify significant binding in core infarction, contralateral hemisphere, and within a defined peri-infarct zone.

Results—Four patients (mean age, 66 years) were imaged across 9 sessions. Four age-matched controls were studied. Within this model, ipsilateral cerebellum was validated as a reference tissue. With the use of control-derived confidence limits and correction for CBV, significant binding potential rises were identified beyond 72 hours and extending to 30 days in core infarction, contralateral hemisphere, and peri-infarct zone.

Conclusions—In ischemic stroke patients, minimal activation of microglia is seen before 72 hours. Beyond this, binding potential rises in core infarction, peri-infarct zone, and contralateral hemisphere to 30 days. This may represent a therapeutic opportunity that extends beyond time windows traditionally reserved for neuroprotection. (*Stroke*. 2006;37:1749-1753.)

Key Words: inflammation ■ microglia ■ positron emission tomography ■ tomography, emission computed

There is strong evidence that neuroinflammation contributes to the pathophysiology of cerebral ischemia and neuronal cell death.¹ A consistent component of this process is recruitment and activation of microglia that produce neurotoxins such as interleukin- 1β (IL- 1β).² IL- 1β in turn promotes neuronal cell death by several mechanisms, including apoptosis.³ Much of this evidence has been obtained from experimental stroke models, although difficulty remains in extrapolation to the clinical setting.⁴ In rat middle cerebral artery (MCA) occlusion models, microglial activation extends beyond core infarction and could contribute to peri-infarct neuronal death.^{5,6} Comparatively little is known regarding whether strategies that seek to downregulate these

processes are likely to provide benefit. In fact, we still do not know whether activated microglia can be demonstrated in peri-infarct areas in clinical stroke, particularly in tissue that may contribute to infarct expansion and poor outcome. Furthermore, we have no clear data on the temporal pattern of microglial activation in this setting. Such information can be obtained only if we have methods of imaging microglial activation in vivo.

The enantiomer (R)-PK11195 (1-2-chlorophenyl-*N*-methyl-*N*-1-methyl-propyl-3-isoquinolone carboxamide) labeled with ^{11}C provides a positron emission tomography (PET) ligand to investigate the role of activated microglia in acute stroke. (R)-PK11195 binds to the peripheral benzodi-

Received November 24, 2005; final revision received January 21, 2006; accepted March 8, 2006.

From the Department of Medicine, University of Cambridge, Addenbrookes Hospital, Cambridge, UK (C.J.S.P.); Wolfson Brain Imaging Centre, University of Cambridge, Addenbrookes Hospital, Cambridge, UK (C.J.S.P., D.W., D.K.M., J.V.G., M.C., T.F., F.A., J.C.B.); Anesthesia Section, Department of Medicine, University of Cambridge, Addenbrookes Hospital, Cambridge, UK (D.K.M.); Department of Neurology, University of Cambridge, Addenbrookes Hospital, Cambridge, UK (C.J.S.P., J.V.G., J.C.B.); and Department of Clinical Neurosciences, Cambridge University Teaching Hospitals Trust, Cambridge, UK (E.A.W.).

Correspondence to C.J.S. Price, Department of Medicine, University of Cambridge, Box 83, R3 Neuroscience, Addenbrookes Hospital, Cambridge, UK. E-mail cp252@medschl.cam.ac.uk

© 2006 American Heart Association, Inc.

Stroke is available at <http://www.strokeaha.org>

DOI: 10.1161/01.STR.0000226980.95389.0b

azepine binding site that is abundant on brain-derived activated microglia. After permanent MCA occlusion in rats, markers of microglial activity increase progressively up to 8 days and extend to 16 days, and binding is observed both in areas of infarction and beyond as defined by histological criteria.⁷ Such binding has been shown to be minimal in normal brain but significantly increased in areas of neuronal damage or inflammation.⁸ Similar evidence of microglial activation can be found with the use of [¹¹C](R)-PK11195 PET in primate models of stroke, in both areas of infarction and peri-infarct tissue.⁹ However, although some clinical PET studies have examined the PK11195 binding after stroke, most patients have been studied on a single occasion, with some limited longitudinal data and information on the spatiotemporal evolution of the microglial response.¹⁰ Few studies have reported PK binding data coregistered with structural imaging such as MRI.

The purpose of this study was to investigate the temporal characteristics of PK binding with serial imaging in the first month after acute ischemic stroke. Our hypothesis, based on the animal literature, was that microglial activity would be present in the subacute phase and that such activity would map to peri-infarct zones, coinciding with areas that are commonly thought of as penumbra.

Subjects and Methods

Patients with clinically defined MCA territory ischemic stroke were studied sequentially within 3 time windows: ≤ 72 hours, 7 to 14 days, and 25 to 30 days (sessions 1 to 3, respectively). Age-matched healthy volunteers were imaged on a single occasion. Exclusion criteria for both patients and volunteers included benzodiazepine exposure within 72 hours and other forms of cerebral pathology. Written informed consent was obtained from all patients and volunteers in accord with protocols approved by the Local Research Ethics Committee and the Administration of Radioactive Substances Advisory Committee.

PET studies were performed sequentially on a GE Advance scanner. After acquisition of an attenuation scan, emission data were acquired for 60 minutes after the intravenous injection of ≈ 250 MBq of [¹¹C](R)-PK11195 (≥ 18.5 GBq/mol specific activity at injection). Approximately 60 minutes after the injection of [¹¹C]PK11195, 750 MBq of C¹⁵O was administered via nasal prongs over 1 minute, and emission data were acquired over the subsequent 5 minutes to quantify cerebral blood volume (CBV). Data were acquired in 3-dimensional mode with corrections for randoms, dead time, normalization, scatter, attenuation, and sensitivity. Total radiation dose per combined session amounted to 2.4 mSv.

Whenever possible, back-to-back T1 coregistration sequence and T2 MRI sequences were performed (Bruker). PET images were coregistered and resliced to the T1-weighted MRI with the use of the mutual information algorithm in SPM2 (Members and Collaborators, 2003). Quantification of [¹¹C]PK11195 binding was made in defined regions of interest on parametric maps of binding potential.

PK parametric binding potential maps were obtained with the use of the simplified reference region (ipsilateral cerebellum) model implemented in the Receptor Parametric Mapping (RPM) software.¹¹ We were concerned that the calculated binding potential would be contaminated by residual intravascular [¹¹C]PK11195 activity that would vary with CBV in the presence of ischemia.¹² The technique used to correct the PK PET data for CBV is described elsewhere.¹³ We measured [¹¹C]PK11195 activity in a vascular region of interest (ROI) defined on the superior sagittal sinus on the C¹⁵O scan to obtain a measure of tracer activity in blood. Parametric CBV images were obtained from the C¹⁵O emission data with the use of standard kinetic models as described elsewhere.¹⁴ These were then used, along with the knowledge of vascular tracer activity, to correct

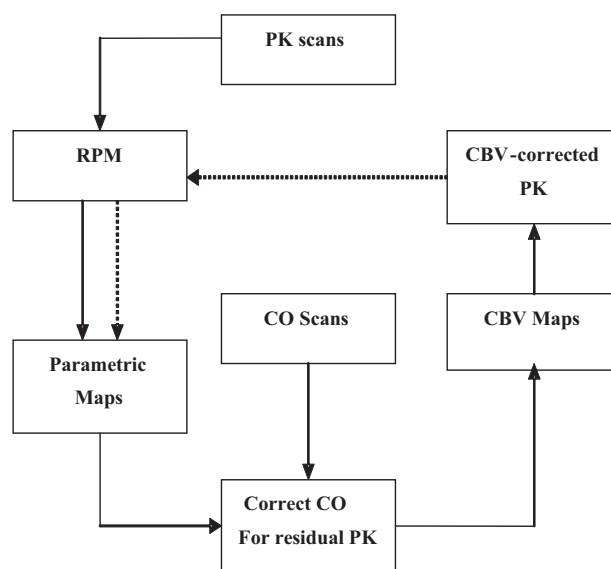


Figure 1. Schematic outlining procedure of generating PK binding potential parametric maps based on CBV correction. RPM indicates regional parametric mapping; CO, carbon monoxide.

images for residual contamination on a voxel basis (Figure 1). Two-tailed paired sample *t* tests were used to test for core stroke ROI-based differences in binding potential with and without CBV correction.

PET images and T2-defined structural core infarct ROIs were coregistered to the chronic T1-weighted images with the use of the vtkCISG software package (Computational Imaging Science Group, 2002). ROIs were defined for core infarct, patient reference tissue (ipsilateral cerebellum), and cerebellum for controls. Core infarct ROI was flipped by symmetry onto the unaffected hemisphere (termed mirror ROI) to allow comparison of findings in the infarcted tissue with those of contralateral homologous territory. MCA ROI was defined in each subject according to previously defined templates and projected onto the subject's normalized binding potential map.¹⁵ Thereafter, each subject's binding potential map was spatially normalized to a T1 template in SPM2 to obtain normalization parameters.

Our choice of reference tissue was dictated by concerns regarding the effects of cerebellar diaschisis.¹⁶ To assess the impact of this phenomenon, linear regression analysis was performed between the individual cerebellar time-activity curves (TACs) of the patients and the mean cerebellar TACs of the controls. To evaluate the suitability of a patient's ipsilateral cerebellum as a reference tissue, comparisons were performed with the use of χ^2 tests between the TACs obtained from the cerebellar region of patients and those obtained from the control subjects. We used the Student *t* test to compare CBV values between patients and controls. Statistical significance was accepted if probability value was <0.05 .

We used the MCA ROI defined above to identify the CIs for PK binding potential values in the normal voxels in control subjects. The 99% upper confidence limit for normal PK binding potential was defined by the 8 MCA ROIs obtained from 4 controls subjects with the use of a 1-tailed *t* test. We wished to define a peripheral ROI that comprised those parts of the MCA territory that were not involved by the infarct. To avoid contamination of this peripheral ROI by infarcted tissue, we dilated the infarct core ROI by 2 PET spatial resolutions to form a "dilated core" ROI. As a marker of the peri-infarct zone, the dilated core ROI was subtracted from the MCA ROI to produce the peripheral ROI (Figure 2). The number of total voxels in an ROI (N_T) and the number of voxels in which PK binding potential intensities were greater or equal to the upper limit of the 99% CI of PK binding potential values from control ROIs ($N_{>UL}$) were measured. In ipsilateral and mirrored peripheral ROIs, ratios of $N_{>UL}/N_T$ were calculated. Ipsilateral peripheral ROI values for $N_{>UL}/N_T$ were

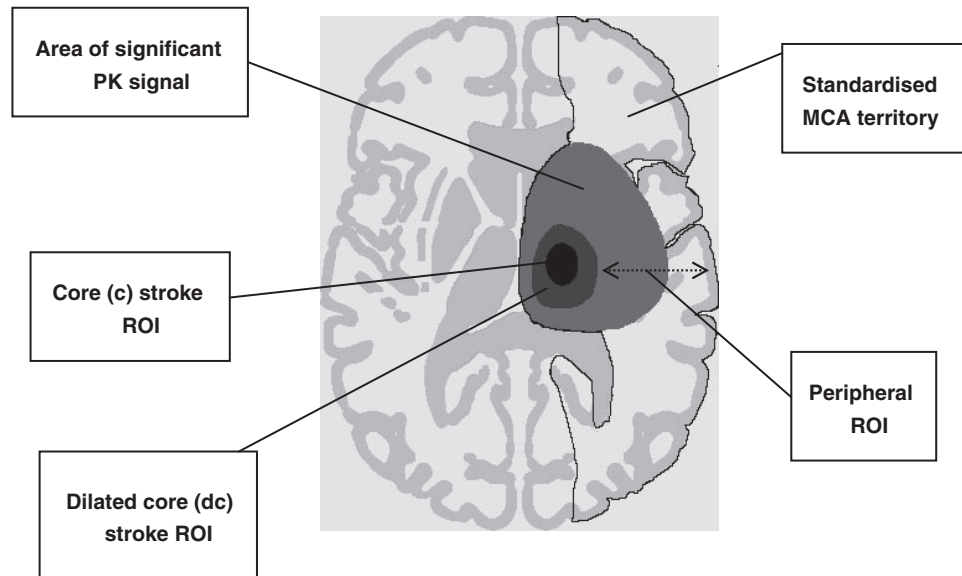


Figure 2. Schematic demonstrating defined ROIs (core, dilated core, peripheral) and MCA used in the image analysis.

compared with values from dilated core and mirrored ROI values on a sessional basis with the use of a paired sample *t* test.

Results

Patients

Four patients (3 male, 1 female) were imaged in a total of 9 sessions. Patient data are given in Table 1. Four age-matched healthy volunteers (aged 64 ± 5 years) were imaged on a single occasion.

Cerebellar TACs

There was a high degree of correlation between the mean TAC in control cerebellar ROIs and that obtained from all ipsilateral patient cerebellar ROIs ($r=0.95$ to 0.99). However, the TAC from the contralateral cerebellar ROI was significantly different from the mean control TAC for 2 patient sessions (patient 1, sessions 1 and 3, $R^2=0.93$ and $R^2=0.91$ and $\chi^2=11.6$, $P=0.04$ and $\chi^2=29.7$, $P<0.01$, respectively). These results confirm the suitability of ipsilateral cerebellum as a reference region for the stroke patient study.

Corrected PK Binding Potential Maps

A 99% upper limit of 0.295 was defined for binding potential from the control data. Significant differences between binding potential values with and without CBV correction were

not observed in patients ($P=0.48$) (Table 2). Mean $N_{>UL}/N_T$ ratios from dilated core ROIs and ipsilateral and contralateral peripheral ROIs are shown in Figure 3. Mean ipsilateral values of $N_{>UL}/N_T$ were not significantly greater than contralateral values on a paired sessional basis ($P=0.57$, $P=0.24$, and $P=0.43$ for sessions 1, 2, and 3, respectively). Sessional differences between ipsilateral mean binding potential in dilated core versus peripheral ROI approached significance in session 2 only ($P=0.27$, $P=0.09$, and $P=0.3$ for sessions 1, 2, and 3, respectively). Images from patient 1 are presented in Figure 4 as an illustration.

Discussion

This image-based study confirms the effective use of PET with the ligand (R)-PK11195 in the study of in vivo spatial and temporal patterns of intrinsic microglial activity in subacute ischemic stroke patients. This study is the first to sequentially and quantitatively study such patterns within this time frame and to do so with the use of correction for CBV.

TABLE 2. Mean PK Binding Potential (Unitless) in Core Stroke ROI With and Without CBV Correction

Patient Session	PK Binding Potential Without CBV Correction	PK Binding Potential With CBV Correction	Absolute Change (% Change)
P1/S1	-0.15 ± 0.17	-0.17 ± 0.18	-0.02 (–13)
P1/S2	2.68 ± 0.51	3.07 ± 0.67	0.39 (13)
P1/S3	0.69 ± 0.4	0.75 ± 0.48	0.07 (9)
P2/S1	-0.09 ± 0.11	-0.08 ± 0.15	0.01 (11)
P2/S2	0.26 ± 0.24	0.24 ± 0.26	-0.02 (–8)
P2/S3	0.25 ± 0.23	0.18 ± 0.24	-0.07 (–28)
P3/S1	-0.09 ± 0.14	-0.07 ± 0.14	0.02 (11)
P4/S1	-0.14 ± 0.34	-0.16 ± 0.2	-0.02 (–14)
P4/S2	0.24 ± 0.22	0.20 ± 0.26	-0.04 (–17)

Values are mean \pm 1SD. Shown are patients (P) 1 to 4, sessions (S) 1 to 3, and absolute and percent differences; $P=0.48$.

TABLE 1. Patient Clinical Summary Including Age, Vascular Territory, National Institutes of Health Stroke Scale Score on Admission, and Time Points of Combined MRI and PET Scans

Patient No.	Age, y	Vascular Territory	Admission NIHSS Score	Combined PET and MRI Image Time, d
1	43	Left MCA	19	2, 13, 30
2	70	Left MCA	13	2, 8, 25
3	77	Left MCA	9	3
4	75	Left MCA	11	2, 10

NIHSS indicates National Institutes of Health Stroke Scale.

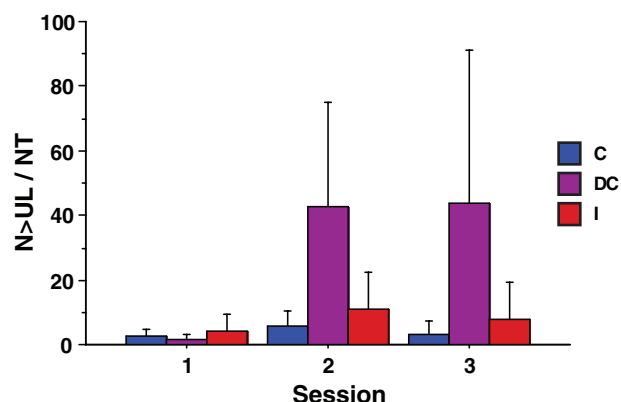


Figure 3. Cell plot of mean percent N_{UL}/N_T (± 2 SE) across 3 time-based sessions for ipsilateral (red) and contralateral (blue) peripheral ROIs and that of dilated core (DC, purple). Control values were nominally 1%.

Although correction for CBV did not significantly influence binding potential overall, given the plasma binding profile of PK11195,¹⁷ it remains likely that wide variations in CBV may influence local binding potential patterns. We have additionally validated the use of ipsilateral cerebellum within the well-established reference tissue model. This model can be used to quantify peri-infarct microglial activation beyond time windows traditionally reserved for neuroprotection.

Dependent on an experimental ischemic model, PK binding is evident from day 1 and may persist until day 16, and this can be confirmed histologically.^{7,18,19} Our data are broadly consistent with this time scale. The data on the temporal characteristics of the response show minimal binding within 72 hours, rising significantly within a week before some reduction by weeks 3 to 4. Spatially, binding remains high within core but appears not to extend to peri-infarct zones until 7 to 10 days. Remote microglial activation extends to contralateral hemisphere at these later time points. In patient-based imaging studies, nonsequential data have been collected at later time points up to 150 days after stroke.¹⁰ Here areas of PK binding can be found extending beyond the infarct site into connected areas of the hemisphere and contralateral thalamus.²⁰ This is thought to represent Wallerian degeneration as a consequence of focal damage.²¹ We found some evidence of this within our mirrored ROIs, primarily at later time points. It remains to be established whether these remote phenomena are related to diaschisis or have other functional significance.

A comparison of these data with an MR-based study using supermagnetic iron oxide particles suggests hematogenous uptake of macrophages within parenchyma within a similar time frame but independent of blood-brain barrier function.²² It is generally thought that postischemic inflammation contributes to ischemic damage by a multitude of mechanisms.^{1,4} The benefi-

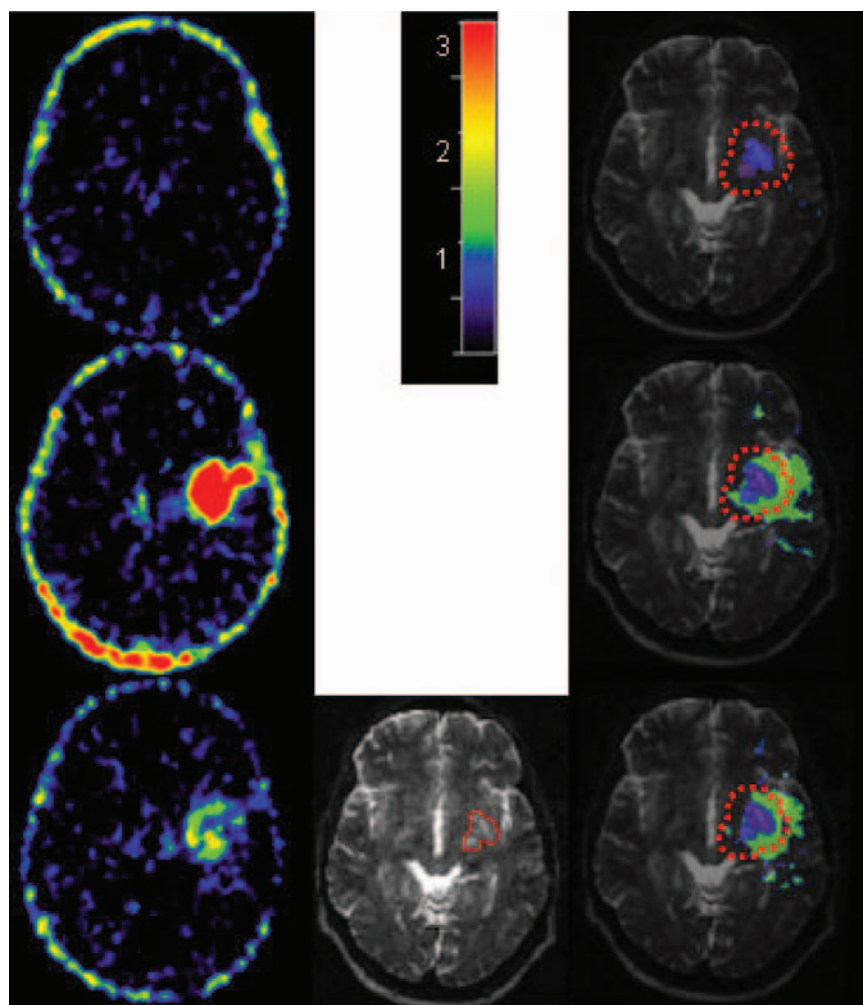


Figure 4. Radiological format binding potential maps (left column) in sessions 1 to 3 (shown top to bottom, respectively) presented for patient 1 alongside structural coregistered T2 MR showing core infarct ROI (middle column). A color BP scale referring to left and middle columns is provided. The right-hand column demonstrates significant binding potential binding within core ROI (blue), dilated core ROI (dotted red), and peripheral ROI (pale green).

cial effects of such a cellular inflammatory response remain unknown but should not be dismissed. Activated microglia possess the potential to produce neurotoxins (including reactive oxygen species and toxic prostanoids) in addition to apoptotic agents such as IL-1 β . In this small study, it is not possible to say whether the magnitude of the microglial response varies according to lesion size or ischemic severity. Correlation with final infarct volume and outcome also remains to be established. A recent study of neutrophil migration in ischemic stroke suggests that there is a heterogeneous response across ischemic strokes of similar type,²³ of which microglia appear to form only a part. Given the ongoing paucity of effective neuroprotective strategies in stroke,²⁴ the detection of microglial activity at later time points when patients present to physicians may provide a target for novel therapeutic agents designed to limit late neuronal damage and improve outcome. Such strategies should be undergone trials alongside existing treatments aimed at restoration of cerebral blood flow, in addition to well-accepted secondary prevention management.

Acknowledgments

The authors thank Diana J. Day, RN, for help with patient recruitment and imaging studies, and Dr J.C. Clark and Prof J.D. Pickard and the radiographers at the Wolfson Brain Imaging Centre for their help in this project.

Sources of Funding

This work was supported by Medical Research Council (UK) grant no. G0001219 to J.C.B. The Stroke Association supports E.A.W. C.J.S.P. was supported on a MRC Clinical Training Fellowship, and both C.J.S.P. and J.V.G. were in receipt of a Raymond and Beverley Sackler Fellowship.

References

- Danton GH, Dietrich WD. Inflammatory mechanisms after ischemia and stroke. *J Neuropathol Exp Neurol*. 2003;62:127–136.
- Yrjanheikki J, Tikka T, Keinänen R, Goldsteins G, Chan PH, Koistinaho J. A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. *Proc Natl Acad Sci U S A*. 1999;96:13496–13500.
- Rothwell N. Interleukin-1 and neuronal injury: mechanisms, modification, and therapeutic potential. *Brain Behav Immun*. 2003;17:152–157.
- Price CJ, Warburton EA, Menon DK. Human cellular inflammation in the pathology of acute cerebral ischaemia. *J Neurol Neurosurg Psychiatry*. 2003;74:1476–1484.
- Zhang Z, Chopp M, Powers C. Temporal profile of microglial response following transient (2 h) middle cerebral artery occlusion. *Brain Res*. 1997;744:189–198.
- Mabuchi T, Kitagawa K, Ohtsuki T, Kuwabara K, Yagita Y, Yanagihara T, Hori M, Matsumoto M. Contribution of microglia/macrophages to expansion of infarction and response of oligodendrocytes after focal cerebral ischemia in rats. *Stroke*. 2000;31:1735–1743.
- Dubois A, Benavides J, Peny B, Duverger D, Fage D, Gotti B, MacKenzie ET, Scatton B. Imaging of primary and remote ischaemic and excitotoxic brain lesions: an autoradiographic study of peripheral type benzodiazepine binding sites in the rat and cat. *Brain Res*. 1988;445:77–90.
- Banati RB, Myers R, Kreutzberg GW. PK ('peripheral benzodiazepine')-binding sites in the CNS indicate early and discrete brain lesions: microautoradiographic detection of [3H]PK11195 binding to activated microglia. *J Neurocytol*. 1997;26:77–82.
- Sette G, Baron JC, Young AR, Miyazawa H, Tillet I, Barre L, Traverre JM, Derlon JM, MacKenzie ET. In vivo mapping of brain benzodiazepine receptor changes by positron emission tomography after focal ischemia in the anesthetized baboon. *Stroke*. 1993;24:2046–2057; discussion 2057–2058.
- Gerhard A, Schwarz J, Myers R, Wise R, Banati RB. Evolution of microglial activation in patients after ischemic stroke: a [11C](R)-PK11195 PET study. *Neuroimage*. 2005;24:591–595.
- Gunn RN, Lammertsma AA, Hume SP, Cunningham VJ. Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. *Neuroimage*. 1997;6:279–287.
- Marchal G, Benali K, Iglesias S, Viader F, Derlon JM, Baron JC. Voxel-based mapping of irreversible ischaemic damage with PET in acute stroke. *Brain*. 1999;122 (pt 12):2387–2400.
- Wang D, Price CJS, Green H, Menon DK, Cleij M, Aigbirhio FI, Warburton EA, Baron JC. CBV correction of 11C-PK11195 binding potential in ischaemic stroke using simplified reference models. *Neuroimage*. 2004;22(suppl 2):96–97.
- Sette G, Baron JC, Mazoyer B, Levasseur M, Pappata S, Crouzel C. Local brain haemodynamics and oxygen metabolism in cerebrovascular disease: positron emission tomography. *Brain*. 1989;112(pt 4):931–951.
- Phan TG, Donnan GA, Wright PM, Reutens DC. A digital map of middle cerebral artery infarcts associated with middle cerebral artery trunk and branch occlusion. *Stroke*. 2005;36:986–991.
- Baron JC, Boussier MG, Comar D. Crossed cerebellar diaschisis in human supratentorial brain infarction. *Ann Neurol*. 1980;8:128.
- Lockhart A, Davis B, Matthews JC, Rahmoune H, Hong G, Gee A, Earnshaw D, Brown J. The peripheral benzodiazepine receptor ligand PK11195 binds with high affinity to the acute phase reactant α 1-acid glycoprotein: implications for the use of the ligand as a CNS inflammatory marker. *Nucl Med Biol*. 2003;30:199–206.
- Benavides J, Capdeville C, Dauphin F, Dubois A, Duverger D, Fage D, Gotti B, MacKenzie ET, Scatton B. The quantification of brain lesions with an omega 3 site ligand: a critical analysis of animal models of cerebral ischaemia and neurodegeneration. *Brain Res*. 1990;522:275–289.
- Schroeter M, Jander S, Huitinga I, Witte OW, Stoll G. Phagocytic response in photochemically induced infarction of rat cerebral cortex: the role of resident microglia. *Stroke*. 1997;28:382–386.
- Pappata S, Levasseur M, Gunn RN, Myers R, Crouzel C, Syrota A, Jones T, Kreutzberg GW, Banati RB. Thalamic microglial activation in ischemic stroke detected in vivo by PET and [11C]PK1195. *Neurology*. 2000;55:1052–1054.
- Myers R, Manjil LG, Frackowiak RS, Cremer JE. [3H]PK 11195 and the localisation of secondary thalamic lesions following focal ischaemia in rat motor cortex. *Neurosci Lett*. 1991;133:20–24.
- Saleh A, Schroeter M, Jonkmann C, Hartung HP, Modder U, Jander S. In vivo MRI of brain inflammation in human ischaemic stroke. *Brain*. 2004;127(pt 7):1670–1677.
- Price CJ, Menon DK, Peters AM, Ballinger JR, Barber RW, Balan KK, Lynch A, Xuereb JH, Fryer T, Guadagno JV, Warburton EA. Cerebral neutrophil recruitment, histology, and outcome in acute ischemic stroke: an imaging-based study. *Stroke*. 2004;35:1659–1664.
- Wahlgren NG, Ahmed N. Neuroprotection in cerebral ischaemia: facts and fancies: the need for new approaches. *Cerebrovasc Dis*. 2004;17(suppl 1):153–166.

Intrinsic Activated Microglia Map to the Peri-infarct Zone in the Subacute Phase of Ischemic Stroke

Christopher J.S. Price, Dechao Wang, David K. Menon, Joe V. Guadagno, Marcel Cleij, Tim Fryer, Franklin Aigbirhio, Jean-Claude Baron and Elizabeth A. Warburton

Stroke. 2006;37:1749-1753; originally published online June 8, 2006;

doi: 10.1161/01.STR.0000226980.95389.0b

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Copyright © 2006 American Heart Association, Inc. All rights reserved.

Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://stroke.ahajournals.org/content/37/7/1749>

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the [Permissions and Rights Question and Answer](#) document.

Reprints: Information about reprints can be found online at:
<http://www.lww.com/reprints>

Subscriptions: Information about subscribing to *Stroke* is online at:
<http://stroke.ahajournals.org/subscriptions/>