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Assessing inflammation in acute intracerebral hemorrhage with PK11195 PET and dynamic contrast-enhanced MRI

~~Imaging inflammation in acute intracerebral hemorrhage: a combined [^{11}C] (*R*) PK11195 PET and DCE-MRI study~~

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Running title: Multimodality imaging of brain inflammation in ICH

Key words: Intracerebral haemorrhage, inflammation, blood-brain barrier, magnetic resonance imaging, positron emission tomography

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Abstract

Background and purpose:

Studies in animal models ~~have suggested~~suggest that inflammation is a major contributor to secondary injury after intracerebral haemorrhage (ICH). Direct, non-invasive monitoring of inflammation in the human brain after ICH will facilitate early-phase development of anti-inflammatory treatments. We sought to investigate the feasibility of multi-modality brain imaging in subacute ICH.

Methods:

Acute ICH patients were recruited to undergo multiparametric ~~magnetic resonance imaging~~MRI (including dynamic contrast enhanced measurement of blood-brain barrier transfer constant (K^{trans}) and ~~positron emission tomography (~~PET~~)~~ with [^{11}C]-(*R*)-PK11195. [^{11}C]-(*R*)-PK11195 binds to the translocator protein 18 kDa (TSPO), which is rapidly upregulated in activated microglia. Circulating inflammatory markers were measured at the time of PET.

Results:

Five patients were recruited to this feasibility study with imaging ~~performed~~ between 5 and 16 days after onset. Etiologies included hypertension-related small vessel disease, cerebral amyloid angiopathy (CAA), cavernoma and arteriovenous malformation (AVM). [^{11}C]-(*R*)-PK11195 binding was low in all hematomas and two (patient 2 (probable CAA) and ~~patient~~ 4 (AVM)) showed widespread increase in binding in the perihematomal region vs. contralateral. All had increased K^{trans} in the perihematomal region (mean difference = 2.2 x 10^{-3} min^{-1}; SD = 1.6 x 10^{-3} min^{-1}) vs. contralateral. Two cases (patients 1 (cavernoma) and 4

(AVM)) had delayed surgery (three and 12-months post-onset, respectively) with biopsies showing intense microglial activation in perilesional tissue.

Conclusions:

Our study demonstrates for the first time the feasibility of performing complex multi-modality brain imaging for non-invasive monitoring of neuroinflammation for this severe stroke subtype. ~~These techniques will be useful tools in developing anti-inflammatory treatments for clinical ICH.~~

Introduction

After intracerebral hemorrhage (ICH), extravasation of blood leads to immediate physical tissue injury. Secondary damage ensues over hours and days, mediated by a cascade of molecular and cellular events involving the toxic effects of blood components and sterile inflammation.[1] ~~[1].~~ Within hours of onset, microglia become activated taking on a pro-inflammatory phenotype, releasing cytokines and chemokines that activate astrocytes and endothelial cells causing blood-brain barrier (BBB) breakdown (alongside the direct effects of the ICH), recruitment of circulating leukocytes, and exacerbation of perihematomal edema.[2] ~~[2].~~ Previous clinical studies in ICH have largely focused on peripheral inflammatory markers showing associations between fever, elevated white blood cell count, interleukin-6 (IL-6), C-reactive protein (CRP), and fibrinogen on admission and worse sub-acute and long-term outcomes.[3] ~~[3].~~ These clinical studies demonstrate the importance of the systemic inflammatory response, but provide no information on processes within the brain, where inflammation contributes directly to secondary injury. Given the growing interest in modulating inflammation in acute ICH and the failure in ischemic stroke to translate findings from animal models, a means of studying inflammation in the intact human brain after ICH is urgently required.

Using advanced multimodality imaging, it is possible to estimate the extent and distribution of microglial activation and BBB breakdown. When activated, microglia express the translocator protein 18 kDa (TSPO), normally present at very low levels in the central nervous system. [^{11}C]-(*R*)-PK11195 binds to TSPO and has been used for ~~positron emission tomography (~~PET~~)~~ studies of acute ischemic stroke,[4] ~~[4],~~ but *in vivo* imaging of microglial activation in ICH has not been previously described. Using ~~magnetic resonance imaging (~~MRI~~)~~ and ~~computed tomography (~~CT~~)~~ dynamic contrast enhanced (DCE) techniques, two previous studies have quantified BBB breakdown after ICH.[5,6] ~~[5, 6].~~ Combining this

technique with [^{11}C]-(*R*)-PK11195 PET will provide a more complete picture of the inflammatory response and allow an understanding of the extent that BBB breakdown co-localizes with microglial activation with important implications for the delivery of an anti-inflammatory drug to the brain. Our aim was to demonstrate the feasibility of combining these techniques in patients during the acute stage of ICH and describe hypothesis-generating preliminary findings.

Methods

Patients between 4 and 28 days after onset of acute, spontaneous ICH were recruited from Salford Royal NHS Foundation Trust (Salford, UK) between 05/12/12 and 28/03/2014, following appropriate approvals. We excluded patients if they had a contraindication to MRI, were pregnant or breast feeding, had significant renal impairment, had an acute neurosurgical procedure performed or planned or who were taking medications which were likely to interfere with [^{11}C]-(*R*)-PK11195 binding. MRI was performed on a Philips 3 T Achieva scanner (Salford Royal Hospital) with an 8 channel head coil. PET scans were performed within 4 days of MRI on a High Resolution Research Tomograph (Siemens/CTI) PET scanner (Wolfson Molecular Imaging Centre, University of Manchester). Venous blood was collected at recruitment (where possible) and immediately prior to the PET scan for measurement of key inflammatory mediators (CRP, IL-6, interleukin-1 (IL-1); see online supplement for immunoassay methods).

MRI included T_1-weighted volumetric Fast Field Echo (T_1-FFE) imaging, T_2-weighted fluid attenuated inversion recovery (FLAIR) imaging and T_1-weighted DCE-MRI. Parametric maps of the blood-brain barrier transfer constant (K^{trans}) were generated from DCE-MRI data using an uptake model (details of MRI acquisition and analysis in online supplementary

methods). PET data were analyzed as previously described.[7] ~~[7]~~ In brief, iterative ordered subset expectation maximization 3D method was used to reconstruct a quantitative series of dynamic images from the 60 min PET emission scan. A reference tissue input function was extracted from cerebellar grey matter in order to generate parametric maps of binding potential BP_{ND} using the simplified reference tissue model. Using SPM (version 8), all maps and images were co-registered to the T_1-weighted volume MRI. The T_1-weighted volume image was segmented into grey matter and white matter probability maps and a maximum probability brain atlas[8] ~~[8]~~ was warped in to individual space. Two regions of interest (ROI) were defined manually from the T_1-weighted and FLAIR images, representing hematoma and perihematomal edema. Corresponding ROIs in the contralateral brain region were generated by flipping the ipsilateral ROIs about the midline in the axial plane excluding any non-brain tissue. The mean binding potential (BP_{ND}) of [^{11}C]-(*R*)-PK11195 and K^{trans} within each ROI was then extracted from the parametric maps using Analyze version 12.0 (Mayo Clinic).

Results

Five patients with acute ICH underwent research brain imaging between 5 and 25 days after onset as part of this feasibility study (Table 1). All patients tolerated the scans without difficulty except patient 3, who was unable to complete the last 22 min of the 60 min emission PET scan and was the only one to undergo both scans on one day. Etiologies confirmed using appropriate clinical imaging included a cavernous angioma, an arteriovenous malformation (Figure 1), probable cerebral amyloid angiopathy and small vessel disease due to chronic hypertension. [^{11}C]-(*R*)-PK11195 binding was low in all hematomas. Patients 2 & 4 showed increased [^{11}C]-(*R*)-PK11195 binding both within the perihematomal edema volume ~~(0.24 and 0.06 respectively, vs. contralateral)~~ and the ipsilateral brain region (~~0.13 &~~

~~0.12 respectively, vs. contralateral;~~ Figure 2). Patients 1 and 4 underwent surgery at 3 and 12 months, respectively, with perlesional tissue demonstrating intense microglial activation.

Analyses of DCE-MRI data show increases in K^{trans} in the perihematomal edema volume (mean difference = 2.2 x 10^{-3} min^{-1}; SD = 1.6 x 10^{-3} min^{-1}) in all 5 patients relative to the contralateral. Visual inspection of images (Figure 2) consistently demonstrates a clear ring of increased K^{trans} adjacent to the outer border of the hematoma. No pattern is suggested for a relationship between circulating inflammatory markers and imaging. ~~, though for all cases, CRP was lower at the time of PET scanning than at recruitment.~~

Discussion

Our small study is the first to show imaging of microglial activation with PET after ICH and demonstrates the feasibility of performing complex multimodality brain imaging after acute ICH. However, we found that performing PET and MR scans on different days may improve successful study completion. All patients show clear ring-shaped BBB breakdown in the outer border of the hematoma, suggesting that delivery of treatments with limited transfer across the intact BBB may be enhanced in acute ICH. We observed a heterogeneous pattern of [^{11}C]-(*R*)-PK11195 binding, with only two patients demonstrating enhanced binding around the hematoma. In neither case was the binding closely co-located with the BBB disruption, suggesting that factors other than microglial activation may drive BBB breakdown after ICH, an observation that should be investigated further in future studies. Late perilesional biopsies in two patients did show intense microglial activation, but only one had enhanced [^{11}C]-(*R*)-PK11195 binding acutely. The long delay between PET and biopsies makes it difficult to interpret this disparity.

Summary/Conclusions

We have demonstrated the feasibility of performing complex multimodality imaging to track the inflammatory response after ICH. This will be a vital tool in investigating this promising therapeutic target with potential use in early phase proof-of-concept clinical trials.

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Table 1. Baseline characteristics, inflammatory markers and imaging data.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient
Baseline Characteristics					
Age (years)	30	63	85	43	46
Sex	Female	Male	Female	Male	Male
GCS at presentation	14	15	15	14	15
ICH etiology	Cavernous angioma[2]	Probable CAA[1]	Primary undetermined	Arteriovenous malformation[2]	Chroni hypertension
ICH location	Right temporal	Right occipital	Left cerebellar	Left parietal	Left lentiform
ICH volume (ml)	6.1	6.7	8.5	30.0	7.1
PHE volume (ml)	4.3	6.9	5.2	15.9	4.4
PET scan (days post-onset)	7	11	25	16	10
MR scan (days post-onset)	9	10	25	12	7
Inflammatory mediators					
IL-1β					
baseline (pg/ml)	-	-	0.56	0.63	0.81
PET scan (pg/ml)	0.81	1.07	1.90	1.94	2.01
IL-6					
baseline (pg/ml)	-	-	7.77	2.35	2.73
PET scan (pg/ml)	3.82	<0.012	4.29	1.5	4.47
IL-8					
baseline (pg/ml)	-	-	16.97	16.12	35.16
PET scan (pg/ml)	11.53	4.84	15.77	10.66	25.59
CRP					
baseline (mg/l)	-	-	2.80	4.32	4.54
PET scan (mg/l)	0.472	0.598	0.862	0.85	1.64
DCE-MRI parameters					
Mean K^{trans} (x 10^{-3} min^{-1})					
Perihematomal edema					
Ipsilateral	1.8	5.7	2.1	2.9	2.2
Contralateral	0.4	0.7	1.0	1.0	0.6
Ipsilateral - contralateral	1.4	5.0	1.0	1.9	1.6

~~Table 1: Baseline characteristics, inflammatory markers and imaging data.~~ [1]Based on modified Boston criteria; [2]Confirmed on histology at later surgical resection. ICH = intracerebral haemorrhage, pg = picogram, ml = millilitre, l = litre, mg = milligram, GCS =

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Glasgow Coma Scale, PHE = peri-haematomal edema, CRP = C-reactive protein, DCE = dynamic contrast-enhanced, K^{trans} = volume transfer constant, IL = interleukin, CAA = cerebral amyloid angiopathy.

Figure legends

Figure 1: Cluster of differentiation 68~~CD68~~ immunostaining (original magnification x400) demonstrates diffuse microglial activation (2a) in patient 1 (suspected cavernoma) and activated microglia and phagocytic activity (2b) in patient 4 (arteriovenous malformation~~AVM~~).

Figure 2: Representative parametric maps of [^{11}C]-(*R*)-PK11195 PET binding potential (BP_{ND}) (superimposed on to T_1-weighted images) and volume transfer constant (K^{trans}) with Fluid-attenuated inversion recovery (FLAIR) ~~FLAIR~~ images from each patient with regions of interest~~ROIs~~ for hematoma (green) and edema (red). Increased [^{11}C]-(*R*)-PK11195 binding is indicated in patients 2&4 by arrows.

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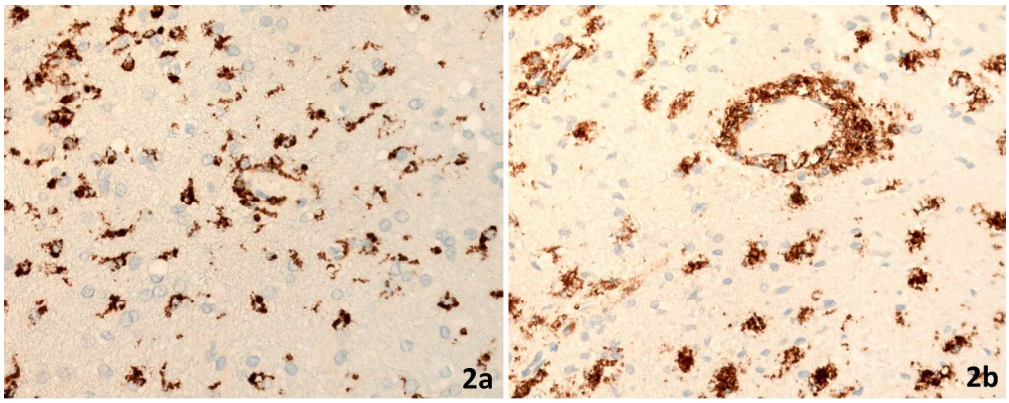

Cluster of differentiation 68 immunostaining (original magnification x400) demonstrates diffuse microglial activation (2a) in patient 1 (suspected cavernoma) and activated microglia and phagocytic activity (2b) in patient 4 (arteriovenous malformation).

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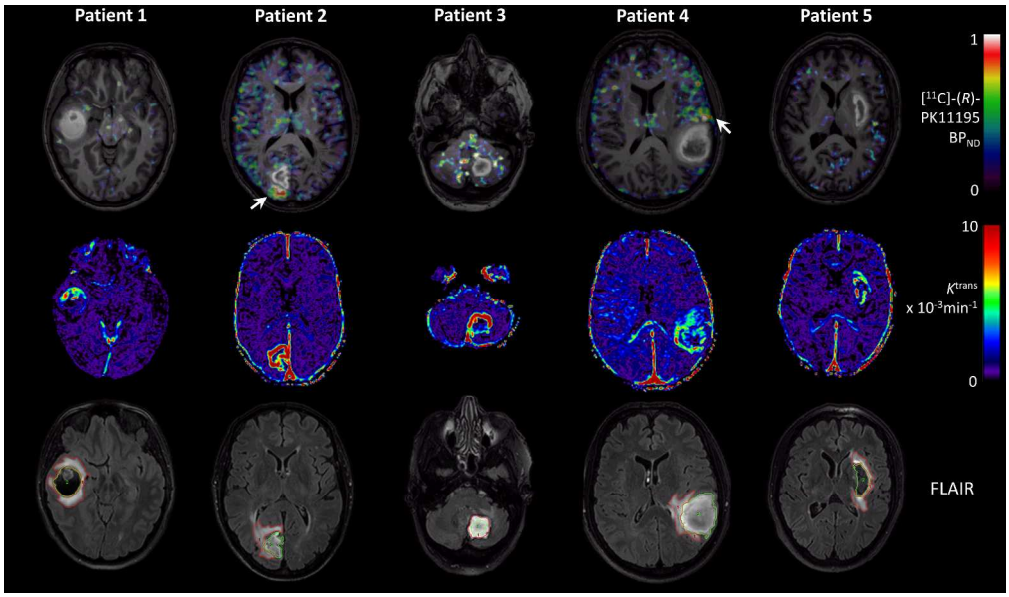

Representative parametric maps of [11C]-(R)-PK11195 PET binding potential (BPND) (superimposed on to T1-weighted images) and volume transfer constant (Ktrans) with Fluid-attenuated inversion recovery (FLAIR) images from each patient with regions of interest for hematoma (green) and edema (red). Increased [11C]-(R)-PK11195 binding is indicated in patients 2&4 by arrows.

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