

Evidence of widespread cerebral microglial activation in amyotrophic lateral sclerosis: an [¹¹C](R)-PK11195 positron emission tomography study

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Received 17 September 2003; revised 24 November 2003; accepted 12 December 2003

Microglial activation is implicated in the pathogenesis of ALS and can be detected in animal models of the disease that demonstrate increased survival when treated with anti-inflammatory drugs. PK11195 is a ligand for the “peripheral benzodiazepine binding site” expressed by activated microglia. Ten ALS patients and 14 healthy controls underwent [¹¹C](R)-PK11195 PET of the brain. Volumes of interest were defined to obtain [¹¹C](R)-PK11195 regional binding potential values for motor and “extra-motor” regions. Significantly increased binding was found in motor cortex ($P = 0.003$), pons ($P = 0.004$), dorsolateral prefrontal cortex ($P = 0.010$) and thalamus ($P = 0.005$) in the ALS patients, with significant correlation between binding in the motor cortex and the burden of upper motor neuron signs clinically ($r = 0.73$, $P = 0.009$). These findings indicate that cerebral microglial activation can be detected in vivo during the evolution of ALS, and support the previous observations that cerebral pathology is widespread. They also argue for the development of therapeutic strategies aimed at inflammatory pathways.

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Keywords: Amyotrophic lateral sclerosis; Motor neuron disease; Microglia; PK11195; Positron emission tomography

Introduction

ALS is a progressive neurodegenerative disease characterised by degeneration of corticospinal, brain stem and spinal cord motor neurons (Rowland and Shneider, 2001). Median survival (in a clinic setting) is 3–4 years from symptom onset (Turner et al., 2002), but can be more than 10 years for a small proportion of

patients (Turner et al., 2003), reflecting the heterogeneous nature of the disease (Shinsuke et al., 2003). The cause of ALS is unknown with the exception of rare familial forms, particularly those associated with mutations of the superoxide dismutase-1 gene (Al-Chalabi and Leigh, 2000; Cleveland and Rothstein, 2001). There is no specific diagnostic test or reliable biological marker to assess disease progression, nor are there any reliable techniques for assessing the presence or extent of cortical pathology. Transcranial magnetic stimulation (Pohl et al., 2001; Schulte-Mattler et al., 1999), MRI (Chan et al., 1999; Ellis et al., 2001) and PET (Abrahams et al., 1996; Lloyd et al., 2000; Turner and Leigh, 2000) have all shown potential in this respect.

The ligand PK11195 (1-[2-chlorophenyl]-*N*-methyl-*N*-[1-methyl-propyl]-3-isoquinolone carboxamide) binds specifically to the “peripheral benzodiazepine binding site” (PBBS). The PBBS is expressed by mitochondria in cells of the mononuclear phagocyte lineage and within the central nervous system is highly expressed by activated, though not resting, microglia—the brain’s intrinsic population of tissue macrophages. Based on the molecular specificity of radioligand–receptor interaction, PET allows the quantitative in vivo functional assessment of specific cellular pathway and their involvement in disease. In combination with volumetric MRI to provide detailed structural information, the entantiomeric PET ligand [¹¹C](R)-PK11195 has been used to measure microglial activation in acute and chronic inflammatory, and non-inflammatory, brain disease (Banati, 2002a).

Microglial cell activation has been implicated in the pathogenesis of several neurodegenerative disorders (McGeer and McGeer, 1998; McGeer et al., 1993). There is evidence that inflammatory mechanisms, in which microglial cells may play a central role, are important mediators of cell death or survival specifically in ALS (Kriz et al., 2002; McGeer and McGeer, 2002). Moreover, drugs aimed at inflammatory pathways have beneficial effects on survival of transgenic mouse models of ALS (Drachman et al., 2002; Zhu et al., 2002). There are also several emerging hypotheses concerning selective motoneuronal cell death, in which microglia may have the

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Available online on ScienceDirect (www.sciencedirect.com.)

central role (Ciesielski-Treska et al., 2001; Raoul et al., 2002; Tortarolo et al., 2003).

In this *in vivo* neuropathological study, [¹¹C](R)-PK11195 PET was used to assess the presence and spatial distribution pattern of microglial activation in the brain in ALS.

Materials and methods

Participants

Sporadic ALS patients fulfilling the categories of “probable” or “definite” ALS according to revised El Escorial criteria (Brooks et al., 1998) were invited to take part in the study. Ten ALS patients (4 female, 6 male; mean age 50 years, range 27–63, SD 13) underwent imaging. Eight of the patients had limb-onset disease, two bulbar-onset, and seven of the 10 patients had bulbar signs at the time of the scan. Subjects were scored at the time of the scan using the revised ALS functional rating scale score (ALSFRS-R) (Cedarbaum et al., 1999). The mean revised ALS functional rating scale score (ALSFRS-R) of the patients was 39. The ALSFRS-R score largely reflects disability due to muscle wasting, that is, lower motor neuron (LMN) involvement, when there is also a recognised spectrum of upper motor neuron (UMN) damage in ALS. To obtain a more parametric scale of UMN involvement (in contrast to the non-parametric El Escorial criteria), patients were also graded in terms of upper motor neuron (UMN) “burden”, by totalling the number of pathological UMN signs on examination (see also Ellis et al., 1999). These were taken as pathologically brisk biceps, supinator, triceps, finger, knee and ankle reflexes, and extensor plantar responses assessed bilaterally and brisk facial and jaw jerks (maximum possible score 16). The mean UMN score was 9 (range 4–16). Disease duration at the time of investigation was calculated in months from date of first symptom onset to date of scan (mean 25 months; range 10–44).

None of the subjects were demented (according to DSM IV criteria), and none were receiving assisted ventilation. Results were compared with those of 14 healthy age-matched volunteers (5 female, 9 male; mean age 58 years, range 32–81, SD 18). There was no concurrent medical illness in any subject and none were taking medication known to have affinity for benzodiazepine binding sites. Informed written consent was obtained, and the study was approved by the Research Ethics Committees of Hammersmith Hospitals and Kings Healthcare NHS Trusts, and The Institute of Psychiatry.

Positron emission tomography

PET was performed with an ECAT 953B PET scanner (CTI/Siemens, Knoxville, TN, USA) operated in 3D acquisition mode. [¹¹C](R)-PK11195 was injected as an intravenous bolus over 30 s at the onset of scanning. The mean injected dose in the ALS patients was 10.3 mCi (range 9.0–11.3, SD 0.7), and in controls 9.8 mCi (range 8.0–11.4, SD 0.9). Dynamic data were collected over 60 min as 18 time frames. A measured attenuation correction was computed with a 15-min transmission scan obtained in 2D mode using an external rotating point source of germanium-68 and acquired before the emission time frames. Scatter was measured and corrected by use of a dual low-energy window method. Data were reconstructed using a ramp filter at Nyquist cut-off producing a reconstructed image resolution of

5.8 mm (full width at half maximum) at the centre of the field of view.

Parametric images of [¹¹C](R)-PK11195 binding potential (BP) were generated with a basis-function implementation of a simplified reference-tissue model (Banati et al., 1999, 2000; Cagnin et al., 2001a; Gunn et al., 1997; Lammertsma and Hume, 1996). This model requires the definition of a reference region or cluster of voxels devoid of specific ligand binding to generate an input function. In a disease such as ALS, where neurodegeneration may be widespread throughout the brain, no discrete reference region may exist. Cluster analysis was, therefore, used to define a population of voxels in each subject with ligand uptake kinetics similar to those of normal cortex derived from a control population to provide the reference input function. Voxels in the raw dynamic data of each subject were segmented into 10 clusters distinguished by the shape of their time–activity curves (TACs). The individual patients’ TACs extracted by cluster analysis were then compared with a normalised mean TAC created from the ligand kinetics of the normal cortex in the healthy patients. The cluster TAC suitable to serve as the patient’s reference normal input kinetic was selected by Chi-squared test using a significance level of $P < 0.05$ (Banati, 2002a; Cagnin et al., 2001a; Gunn et al., 1998).

Volumes of interest

Three dimensional T1-weighted magnetic resonance images (1.0 Tesla, Picker HPQ, Ohio, USA) were obtained on each subject. Volumes of interest (VOIs) were drawn bilaterally on consecutive slices of each individuals’ volumetric MRI, before co-registration with the respective PET image volume for blinding purposes (Analyze®Direct Inc., Lenexa, KS) (Fig. 1). To assess the motor system, a VOI was drawn over the pre-central gyrus (motor cortex) (mean volume 9817 mm², SD 2092). A further VOI was drawn over the pons (mean volume 5957 mm², SD 1171).

Five VOIs were chosen for the assessment of possible “extra-motor” involvement—frontal, temporal and occipital lobes, thalamus and basal ganglia. The frontal lobe VOI incorporated the dorsolateral prefrontal region (DLPFC) (mean volume 14380 mm², SD 2786). The temporal VOI sampled the superior temporal gyrus (superior gyrus to ensure incorporation in the PET field of view for all subjects) (mean volume 5231 mm², SD 998). The putamen was chosen as a representative basal ganglia VOI (mean volume 3913 mm², SD 682). The thalamic VOI mean volume was 5031 mm² (SD 694). Finally, a cylindrical VOI was placed centrally within both occipital lobes (3120 mm²). There was no significant difference in the VOI sizes overall between subject groups.

Statistical analysis

The VOIs were positioned onto the parametric [¹¹C](R)-PK11195 PET images, and a mean BP value for [¹¹C](R)-PK11195 for each region in all subjects was generated (Analyze®Direct Inc., Lenexa, KS). Values from right and left regions were combined and averaged in the calculation of the control subject mean BP for each region. However, in the ALS patients, a more conservative analysis was used, maintaining separate mean BP values for left and right hemispheres because of the potential asymmetry of cerebral disease in ALS.

A one-tailed *t* test (testing for ALS increases only), and assuming unequal group variances (more conservative), was carried out using the mean BP values for VOIs in the control and ALS

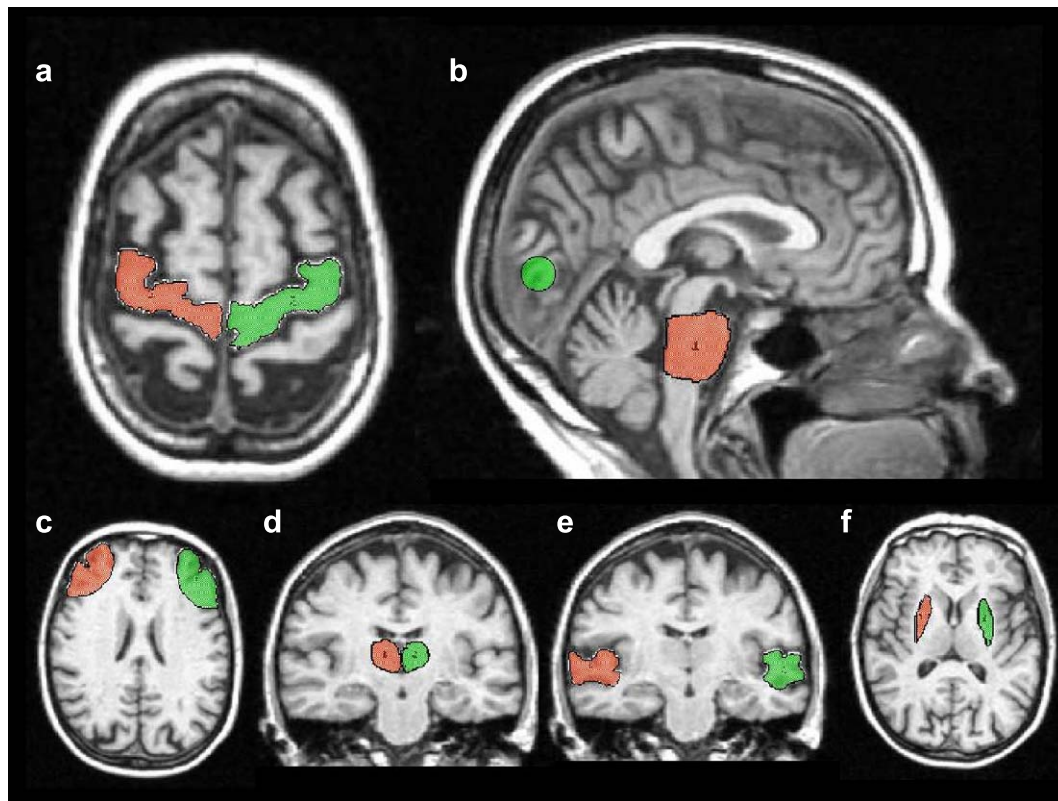


Fig. 1. Volumes of interest (VOIs) drawn for each subject on individual T1-weighted MRIs in the absence of the PET images. The seven regions studied include motor cortex (pre-central gyrus) (a), pons and occipital lobes (b), frontal lobe (dorsolateral prefrontal cortex) (c), thalamus (d), superior temporal lobe (e) and putamen (f).

patient groups, for each of the seven regions. For the pontine VOI analysis, an isolated control patient value was omitted because the 10-cm PET camera axial field of view did not incorporate the pons

in this subject. Bivariate correlations between ALS patient regional BP values and disease duration, ALSFRS-R and UMN score were examined using a Pearson correlation coefficient and one-tailed test

Table 1
[¹¹C](R)-PK11195 binding potential values for all subjects and results of *t* test and bivariate correlation analyses

Subject				Volume of interest													
				MC		PONS		DLPFC		Thalamus		Temporal		Putamen		Occipital	
ALS no.	Disease duration	ALSFRS-R	UMN score	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
Controls	Mean			0.076		0.301		0.111		0.323		0.126		0.215		0.065	
	SD			0.076		0.078		0.058		0.106		0.079		0.084		0.087	
1	26	35	7	0.132	0.170	0.444	0.430	0.176	0.161	0.416	0.458	0.154	0.212	0.231	0.303	0.024	0.079
2	10	32	16	0.275	0.217	0.332	0.439	0.188	0.200	0.452	0.544	0.175	0.201	0.213	0.187	0.063	0.145
3	30	38	4	0.146	0.112	0.275	0.290	0.187	0.123	0.215	0.272	0.129	0.032	0.026	0.174	-0.020	0.022
4	32	37	6	0.072	0.125	0.404	0.358	0.111	0.084	0.374	0.497	0.088	0.149	0.184	0.165	0.092	0.051
5	40	43	7	0.221	0.177	0.331	0.451	0.246	0.312	0.516	0.526	0.140	0.303	0.456	0.137	0.199	0.156
6	11	42	13	0.213	0.189	0.590	0.637	0.243	0.169	0.595	0.519	0.236	0.175	0.420	0.345	0.147	0.101
7	15	41	9	0.105	0.127	0.315	0.426	0.045	0.056	0.287	0.395	0.020	0.126	0.307	0.260	0.098	0.144
8	25	46	4	0.024	0.123	0.375	0.282	0.115	0.081	0.298	0.330	0.088	0.061	0.251	0.178	-0.030	-0.009
9	44	38	11	0.113	0.171	0.324	0.414	0.245	0.207	0.399	0.381	0.152	0.167	0.341	0.246	0.117	0.134
10	20	39	13	0.154	0.143	0.332	0.326	0.162	0.195	0.543	0.451	0.172	0.166	0.238	0.289	0.174	0.057
Mean				0.150		0.389		0.165		0.423		0.147		0.248		0.087	
SD				0.057		0.383		0.165		0.422		0.143		0.245		0.091	
<i>t</i> test (<i>p</i>)				0.003*		0.004*		0.010*		0.005*		0.213		0.153		0.211	
UMN correlation				0.73*		0.41		0.36		0.66*		0.57*		0.47		0.60*	

MC—motor cortex; DLPFC—dorsolateral prefrontal cortex.

**P* < 0.05.

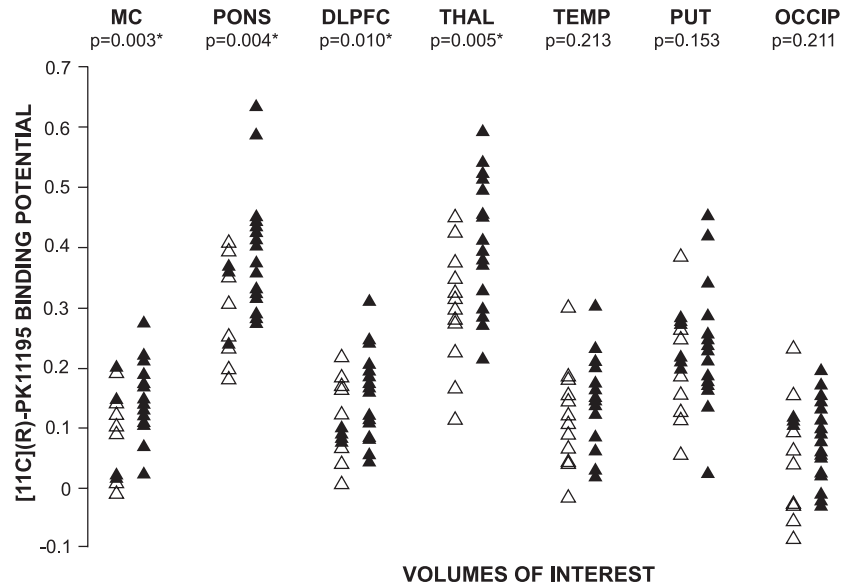


Fig. 2. Mean $[^{11}\text{C}](\text{R})\text{-PK11195}$ binding potential values for all VOIs drawn. Control subject values are in the left column (Δ), and ALS patient values in the right column (\blacktriangle), of each pair. * $P < 0.05$, MC-motor cortex, DLPFC-dorsolateral prefrontal cortex, THAL-thalamus, TEMP-temporal lobe, PUT-putamen, OCCIP-occipital lobe.

of significance (SPSS Inc., Chicago, IL). For this analysis, a single mean BP value for both hemispheres in each region was used in the ALS patient group.

Potential error due to multiple comparisons was examined using the Hochberg correction and p-plot graphical method for

the estimation of the number of “true” null hypotheses (Turkheimer et al., 2001). Analysis using this method indicated that correction was not required for these data.

Results

$[^{11}\text{C}](\text{R})\text{-PK11195}$ BP values for all subjects are summarised in Table 1 (the occasional negative mean BP values represent variation near to zero). Variable binding of $[^{11}\text{C}](\text{R})\text{-PK11195}$ was seen in all regions in both patients and controls with overlap of the ranges of the two groups (Fig. 2). Despite this, significantly increased mean binding of $[^{11}\text{C}](\text{R})\text{-PK11195}$ was demonstrable in the region of the motor cortex ($P = 0.003$), pons ($P = 0.004$) (Fig. 3b), frontal lobe region (DLPFC) ($P = 0.010$) (Fig. 3d), and

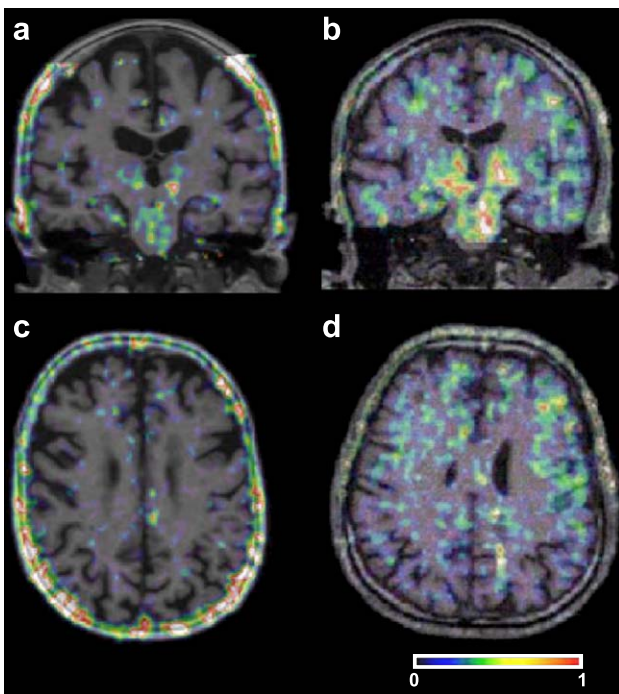


Fig. 3. Coronal and transverse $[^{11}\text{C}](\text{R})\text{-PK11195}$ PET binding potential maps co-registered with T1-weighted MR images in control (a, c) and ALS patients (b, d), demonstrating visibly increased signal in the region of the thalamus and pons (b); and frontal lobe (d). The colour scale is calibrated for $[^{11}\text{C}](\text{R})\text{-PK11195}$ binding.

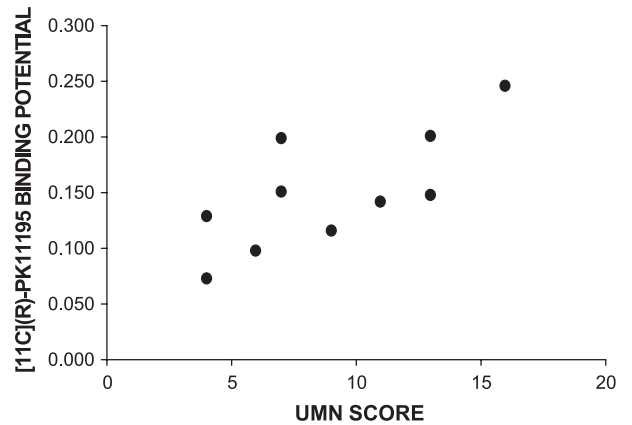


Fig. 4. Scatter plot of mean $[^{11}\text{C}](\text{R})\text{-PK11195}$ binding in the motor cortex of the ALS patients against their UMN score, demonstrating a strong positive correlation ($r = 0.73$, $P = 0.009$).

thalamus ($P = 0.005$) (Fig. 3b) in the ALS subjects. This remained the case ($P < 0.05$) considering left–right summed values for ALS patient BPs, and also after removal of a single outlying ALS patient (no. 6) with atypically high individual values, especially in the pontine region. Binding in the region of the temporal lobes, putamen and occipital lobes were not significantly increased ($P = 0.213$, $P = 0.153$ and $P = 0.211$, respectively).

There was a strong correlation between the individual BP values of the ALS patients and their clinical UMN scores, both for motor cortex ($r = 0.73$, $P = 0.009$) (Fig. 4) and thalamus ($r = 0.66$, $P = 0.018$). No clear difference in the binding of [^{11}C](R)-PK11195 between ALS patients with bulbar- or limb-onset, and no significant correlation with either ALSFRS-R score or disease duration for any region was seen.

Discussion

This [^{11}C](R)-PK11195 PET study provides the first in vivo evidence of diffusely increased microglial activation in both motor and “extra-motor” cerebral regions in a group of ALS patients during the evolution of the disease. The sample is small and the conclusions must therefore be approached cautiously. Although there was considerable individual variability in the ALS BP values for [^{11}C](R)-PK11195, these correlated significantly with clinical UMN “burden”, particularly in the region of the motor cortex.

Peripheral benzodiazepine binding sites in brain disease

Detecting the presence of the PBBS using [^3H]PK11195 (1-[2-chlorophenyl]-*N*-methyl-*N*-[1-methyl-propyl]-3-isoquinolone carboxamide) has become a well-established measure of active disease in the brain (Banati et al., 1997; Benavides et al., 1983; Doble et al., 1987). Particularly abundant in cells of mononuclear-phagocyte lineage, the PBBS is expressed in many organs except the healthy brain. However, an active pathological process leads to the invasion of cells of mononuclear-phagocyte lineage or the activation of microglia—the brain’s intrinsic source of macrophages.

The enantiomeric PET ligand [^{11}C](R)-PK11195 has been used as a sensitive in vivo marker of active disease in a variety of acute and slowly progressive neurological conditions, including stroke (Gerhard et al., 2000; Pappata et al., 2000), herpes encephalitis (Cagnin et al., 2001b), Rassmussen’s encephalitis (Banati et al., 1999), Alzheimer’s disease (Cagnin et al., 2001a), multiple system atrophy (Gerhard et al., 2003) and in multiple sclerosis, where it correlated with disability and neuropathological findings (Banati et al., 2000). High [^{11}C](R)-PK11195 signals are regularly seen in the skull (Figs. 3a and c), particularly in the orbits and sinuses of all subjects as well as low levels of constitutive binding, specifically within the midbrain, pons and thalamus, which increases with age (Banati et al., 2000; Cagnin et al., 2001a). The slightly older mean age of the controls in this study (58 versus 50 years) therefore has the potential to underestimate the size of the group differences. Another caveat in interpreting studies of microglial activation (particularly in post mortem neuropathological studies) is the likelihood, in debilitating conditions, that microglial activation might reflect agonal brain injury. Patients known to be hypoxic were, therefore, not included in this study.

Microglia in amyotrophic lateral sclerosis

Microglial cell activation has been implicated in the pathogenesis of several neurodegenerative conditions including ALS (McGeer and McGeer, 1998; McGeer et al., 1993; Schwab et al., 1996). There is also evidence for a more general inflammatory basis to pathogenesis in ALS (McGeer and McGeer, 2002; Urbanek and Jansa, 1974; Weydt et al., 2002; Wisniewska and Tytuliska, 1966). A post-mortem immunocytochemical study of the motor cortex in ALS demonstrated a central role for microglia (with other cells), in a neuronal loss postulated to be immune-mediated (Troost et al., 1993), and another studied the presence of cerebral microglia specifically in extra-motor regions (Latsoudis et al., 1999). Several immunohistochemical studies have characterised an inflammatory infiltrate in ALS spinal cord (Engelhardt et al., 1993; Troost et al., 1990) and brain (Kawamata et al., 1992). The upregulated expression by microglial cells of macrophage colony stimulating factor has been demonstrated in the brains of ALS patients (Akiyama et al., 1994), and antibodies to microglia and brain macrophages have been found in spinal fluid (Banati et al., 1995). In mouse models of ALS, intense microglial activation has been demonstrated, which increased with disease progression (Alexianu et al., 2001; Hall et al., 1998). Another study noted neurodegenerative changes that preceded microglial activation (Rathke-Hartlieb et al., 1999) suggesting that it was a secondary response to a direct motoneuronal injury (Mariotti and Bentivoglio, 2000). Microglial activation is also inducible by immunoglobulin from ALS subjects injected into mouse spinal cord (Obal et al., 2001).

There are several emerging molecular hypotheses accounting for selective motoneuronal cell death in ALS, in which activated microglia may play a potentially key role. A splice variant of peripherin—a neurofilament protein associated with pathological neuronal aggregates found in ALS (Beaulieu et al., 1999)—is thought to be a potential “tag” for a microglial-induced apoptotic process potentially involved in selective motor neuronal cell death, possibly via tumour necrosis factor alpha (Robertson et al., 2001). The ligand for the apoptotic receptor Fas can trigger motor neuronal cell death, potentiated by the presence of mutant superoxide dismutase-1 (*SOD1*-genetic mutations of which are associated with ALS) (Raoul et al., 2002). Activated microglia have been shown to secrete Fas ligand, suggesting another potential role in pathogenesis (Ciesielski-Treska et al., 2001). Finally, p38 stress-activated kinase is an intracellular molecule of the mitogen-activated protein kinase superfamily, activated in response to a variety of physiological stresses (Kyriakis and Avruch, 2001). Theoretically, it might have a role in the aberrant phosphorylation of neurofilaments, resulting in their pathological accumulation within motor neurons. The co-localisation of an activated p38 isoform with phosphorylated neurofilaments in mutant *SOD1* transgenic mice lends support to this theory (Tortarolo et al., 2003).

Considering therapeutic agents aimed at inflammatory pathways (in which activated microglia might play a central role), there has been interest in the use of both cyclooxygenase inhibitors (Asanuma et al., 2001; Klegeris and McGeer, 2002) and minocycline as neuroprotective agents. Cyclooxygenases are found throughout the central nervous system (Yermakova and O’Banion, 2000). Cyclooxygenase 2 is involved in the synthesis of prostaglandin E2 and possibly the regulation of glutamate release. Marked increases in COX-2 have been demonstrated in ALS

spinal cord (Yasojima et al., 2001). Celecoxib, an inhibitor of cyclooxygenase type 2 (COX-2) and nimesulide, has been shown to have neuroprotective effects in the superoxide dismutase (*SOD1*) transgenic ALS mouse (Drachman et al., 2002; Pompl et al., 2003). The antibiotic minocycline is a poly (ADP-ribose) polymerase (PARP) gamma agonist and is also thought to have broadly anti-inflammatory/apoptotic properties. Several studies in cell culture and mouse models of ALS have demonstrated its ability to reduce microglial activation and delay disease onset or increase survival in the *SOD1* transgenic mouse model of ALS (Kriz et al., 2002, 2003; Tikka et al., 2002; Van Den Bosch et al., 2002; Zhu et al., 2002). Clinical trials of these agents are in progress.

Other studies in amyotrophic lateral sclerosis

Autoradiographic binding of [³H]-PK11195 in ALS has been studied in post-mortem spinal cord tissue (Sitte et al., 2001). Significantly increased binding was found in the descending corticospinal tracts (CST) of the ALS patients compared to controls with a surprising lack of binding within the spinal anterior horns. The evolution of the CST lesion in the sequence of the pathogenic cascade remains a matter of debate. The “dying-back” hypothesis to account for UMN involvement seems logical from the consistent finding of pathology at the spinal anterior horn and the occurrence of LMN—only forms of the disease (Chou and Norris, 1993). However, involvement of the CST is a central feature of ALS, even in clinically lower motor neuron (LMN)—only forms (Ince et al., 2003). Support for a “corticomotoneuronal” or “dying-forward” hypothesis, where the primary event is loss of the UMN (Eisen et al., 1992), is controversial (Swash, 1992). This [¹¹C](R)-PK11195 PET study provides in vivo evidence for increased microglial activation in the motor cortex and pons. These observations are in keeping with the specific requirement for clinical UMN involvement in all of the patients studied (El Escorial “probable” or “definite” ALS), and the correlation demonstrated between UMN “burden” clinically and microglial activation in the motor cortex is highly significant. This finding supports the hypothesis that patients with greater UMN involvement have increased cortical disease, as other imaging studies have shown (Ellis et al., 1999). The lack of correlation with disease duration is not surprising, given the heterogeneity of the condition, and the lack of correlation with the ALSFRS-R probably reflects the fact that functional scales are far more sensitive to lower motor neuron involvement and the resultant muscle wasting or the limited range scores in the patient sample.

This study suggests that there is significant, albeit “low-grade”, microglial activation in the frontal lobes of ALS patients. There is evidence from neuropathological studies (Kushner et al., 1991; Nagy et al., 1994), in vivo imaging research using PET (Abrahams et al., 1996; Kew et al., 1993; Lloyd et al., 2000; Ludolph et al., 1992) and quantitative volumetric MRI (Ellis et al., 2001), suggesting that cerebral pathology extends far beyond the primary motor cortex and corticospinal tracts in ALS. The cognitive abnormalities of ALS are now well characterised (Neary and Snowden, 1996). Although frank dementia (usually frontotemporal in type) is rare in ALS, more subtle cognitive deficits frequently relating to executive function can be reliably identified in up to 50% patients (Abrahams et al., 2000; Massman et al., 1996). These cognitive changes are particularly associated with functional changes in the dorsolateral pre-frontal cortex (Abrahams et al., 1996) and a region of interest incorporating

the DLPFC was, therefore, included in our study. Pathological studies of the distribution of inclusion bodies in frontotemporal dementia compared with ALS patients, demonstrates a common localisation leading to the concept of a spectrum of clinical presentation between the two conditions (Kew and Leigh, 1992). Our findings lend further support to this notion (Lomen-Hoerth et al., 2002).

Regionally increased PK11195 binding does not necessarily imply the presence of amoeboid or phagocytic microglia/brain macrophages, but may also indicate active tissue responses to disconnection, in retrograde, anterograde or trans-synaptic projection areas (Banati, 2002b; Banati et al., 2000). While in these areas, activated microglia do not herald overt destructive tissue pathology, they may render these regions susceptible to secondary recruitment of peripheral blood-borne inflammatory cells as has been demonstrated in models of motoneuron injury (Flugel et al., 2001; Raivich et al., 1998). Such remote activation of microglia may potentially be responsible for secondary progressive brain pathology extending beyond the initially affected neural system.

One example of an area with secondary remote activation of microglia might be in the thalamus. Although concerned with the integration of sensory information, its reciprocal connections with multiple cortical areas may account for the significant microglial activation demonstrated in this study. Indeed, neurodegeneration in the thalamus is noted to be amongst the earliest features of the wobbler mouse model of ALS (Rathke-Hartlieb et al., 1999). Our study also showed significant correlation between thalamic microglial activation and UMN burden clinically, perhaps reflecting its connectivity with motor pathways.

Although changes in mean [¹¹C](R)-PK11195 binding in the region of the temporal lobe and putamen of ALS cases were not significant in this study, this may reflect the small sample and the heterogeneity of the disease. Indeed, the removal of a single outlying control subject with atypically high BP values for both these regions resulted in a trend towards significant binding in both regions ($P = 0.074$ and $P = 0.064$, respectively). Post-mortem studies have identified pathological changes within the temporal lobes of ALS patients (Piao et al., 2003), and other research points to a possible dopaminergic deficit (Borasio et al., 1998; Kostic et al., 1997; Przedborski et al., 1996; Takahashi et al., 1993). Both of these regions might therefore be involved in pathogenesis, and a more sensitive in vivo technique may reveal microglial activation in these and other areas.

Conclusions

This in vivo PET study demonstrates what appears to be a more diffuse microglial activation compared to the greater, more focal binding seen in [¹¹C](R)-PK11195 PET studies of other cerebral diseases. Nonetheless, these findings represent the first evidence that microglial activation is a feature—and not only an end-stage feature—of ALS. This has important implications for our understanding of pathogenic mechanisms, in many of which, a clear potential role for activated microglia is emerging. It also confirms the extension of the disease process in ALS to encompass “extra-motor” areas, including the prefrontal region. Further studies are warranted, particularly to determine whether [¹¹C](R)-PK11195 binding changes as the disease evolves. It will be particularly interesting to study individuals “at risk” of

developing ALS, for example, asymptomatic individuals with known mutations of the *SOD1* gene. Such studies may indicate at what stage in the evolution of clinical symptoms and signs, significant microglial activation occurs. It may also be possible to investigate whether therapeutic agents modify this microglial activity.

Acknowledgments

MRT is supported by a Wellcome Trust Clinical Research Fellowship. The King's MND Care and Research Centre (MRT, CES, PNL) receives support from the Motor Neurone Disease Association (UK). DJB receives support from the Medical Research Council. RBB has received support from the Max-Planck-Institute of Neurobiology (Martinsried, Germany) and the Deutsche Forschungsgemeinschaft grant: "The mitochondrial benzodiazepine receptor as indicator of early CNS pathology, clinical application in PET"; the European Community, and the International Institute for Research in Paraplegia (Zurich, Switzerland). AC was supported by a Fellowship from the European Community in the Training and Mobility of Researchers Programme in Biomedicine.

We thank the radiographers and technicians Andy Blyth, Hope McDevitt, Joanne Holmes, Stella Ahier and Len Schnorr for their help and support and to all the patients who took part in the study.

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