Distinct cerebral lesions in sporadic and ‘D90A’ SOD1 ALS: studies with [11C]flumazenil PET

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Summary

Five to ten percent of amyotrophic lateral sclerosis (ALS) cases are associated with mutations of the superoxide dismutase-1 (SOD1) gene, and the ‘D90A’ mutation is associated with a unique phenotype and markedly slower disease progression (mean survival time 14 years). Relative sparing of inhibitory cortical neuronal circuits might be one mechanism contributing to the slower progression in patients homozygous for the D90A mutation (homD90A). The GABA_A receptor PET ligand [11C]flumazenil has demonstrated motor and extra-motor cortical changes in sporadic ALS. In this study, we used [11C]flumazenil PET to explore differences in the pattern of cortical involvement between sporadic and genetically homogeneous ALS groups. Twenty-four sporadic ALS (sALS) and 10 homD90A patients underwent [11C]flumazenil PET of the brain. In addition, two subjects homozygous for the D90A mutation, but without symptoms or signs (‘pre-symptomatic’, psD90A), also underwent imaging. Results for each group were compared with those for 24 healthy controls of similar age. Decreases in the binding of [11C]flumazenil in the sALS group were found within premotor regions, motor cortex and posterior motor association areas. In the homD90A group of ALS patients, however, decreases were concentrated in the left fronto-temporal junction and anterior cingulate gyrus. In the two psD90A subjects, a small focus of reduced [11C]flumazenil binding at the left fronto-temporal junction was seen, similar to the pattern seen in the clinically affected patients. Within the sALS group, there was no statistically significant association between decreases in cortical [11C]flumazenil binding and revised ALS functional rating scale (ALSFRS-R score), whereas the upper motor neuron (UMN) score correlated with widespread and marked cortical decreases over the dominant hemisphere. In the homD90A group, there was a stronger statistical association between reduced cortical [11C]flumazenil binding and the ALSFRS-R, rather than the UMN, score, and also with disease duration. This study provides evidence for differences in the distribution of reduced cortical [11C]flumazenil binding in homD90A compared with sALS patients. We hypothesize that this might reflect differences in cortical neuronal vulnerability.

Keywords: amyotrophic lateral sclerosis; motor neuron disease; flumazenil; D90A; SOD1

Abbreviations: ALS = amyotrophic lateral sclerosis; ALSFRS-R = revised ALS functional rating scale; homD90A = homozygous for the D90A SOD1 gene mutation; LMN = lower motor neuron; PMA = progressive muscular atrophy; psD90A = pre-symptomatic homozygous for the D90A SOD1 gene mutation; sALS = sporadic ALS; SOD1 = superoxide dismutase-1; SPM = statistical parametric mapping; UMN = upper motor neuron; VD = volume of distribution

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Introduction

Although degeneration of corticospinal tract, brainstem and spinal motor neurons is the pathological hallmark of amyotrophic lateral sclerosis (ALS), there is now evidence from neuropathology (Smith, 1960; Maekawa et al., 2004), neuroimaging (Kew et al., 1993a; Pioro et al., 1994; Abrahams et al., 1996; Lloyd et al., 2000; Ellis et al., 2001; Turner et al., 2004), neurophysiology (Ziemann et al., 1997; Turner et al., 2005) and neuropsychology (Kew and Leigh, 1992; Abrahams et al., 2000; Lomen-Hoerth et al., 2002) supporting a parallel and widespread cortical lesion in ALS.

Five to ten percent of ALS cases are associated with mutations of the superoxide dismutase-1 (SOD1) gene on chromosome 21 (Rosen et al., 2003). Most of the 109 known SOD1 gene mutations are associated with variable phenotypes, a survival period typically in the order of 1–4 years (as for sporadic cases) and are inherited dominantly (Andersen et al., 2003). The D90A SOD1 gene mutation is usually inherited as a recessive trait. Homozygous patients (homD90A) show a characteristic and predictable phenotype beginning with lower limb spasticity and paresis prior to upper limb and bulbar involvement, with slow progression and long survival—averaging 14 years (Andersen et al., 1996). The disease has a high penetrance in homozygotes (Andersen et al., 1997).

Flumazenil binds to the benzodiazepine subunit of the GABA_A receptor present on neurons throughout the cerebral cortex. [11C]Flumazenil PET therefore provides a potential marker for cortical neuronal loss or dysfunction. It may also reflect altered GABAergic inhibitory function, postulated to differ between sporadic ALS (sALS) and homD90A patients (Weber et al., 2000; Turner et al., 2005), and possibly be related to phenotype and disease progression. Using this technique, we studied [11C]Flumazenil binding in sALS and homD90A patients. In addition, we studied two unaffected (‘pre-symptomatic’, psD90A) subjects homozygous for the same mutation in an attempt to identify early cortical changes. We also studied the relationship of functional and clinical measures of disease to cortical involvement in sporadic and homD90A ALS.

Subjects and methods

Participants

All ALS patients were diagnosed according to revised El Escorial criteria (Brooks et al., 2000). A small number of patients initially diagnosed with progressive muscular atrophy (PMA) were also included to provide a broader range of definite upper motor neuron (UMN) involvement (zero for the PMA cases) within the subsequent analysis. All have subsequently shown disease progression consistent with ALS.

Subjects designated as having sALS did not include those known to have mutations of the SOD1 gene, or an established family history of ALS. The psD90A individuals were aware of their genetic status and its implications. Results were compared with healthy controls of similar age. Genetic analysis was performed using DNA extracted from whole blood after written informed consent, and tested by sequencing of all five exons of the SOD1 gene (Rosen et al., 1993).

Disease duration at the time of investigation was calculated for all patients in months from the date of onset of weakness to the date of investigation. ALS patients were rated functionally using the revised ALS functional rating scale (ALSFRS-R) (Cedarbaum et al., 1999). This is a scale from 0 to 48 within 12 categories, where a lower score is associated with increased disability. They were also graded in terms of UMN burden, by summing the number of pathological UMN signs on examination (see Turner et al., 2004).

Subjects were asked to omit all medication, including riluzole where prescribed, for at least 48 h prior to testing. None of the subjects studied was taking any regular medication known to interact with the benzodiazepine receptor, and none was demented (according to DSM-IV criteria). All patients gave informed written consent to undergo PET, and the study was approved by the Administration of Radioactive Substances Advisory Committee (ARSAC), and the Ethics Committees of the Institute of Psychiatry, Hammersmith Hospitals NHS Trust, Umeå University and King’s College Hospitals.

PET image acquisition

[11C]Flumazenil PET images were acquired in 3D mode using an ECAT 953B PET scanner (CTI/Siemens, Knoxville, TN), in 20 frames over 90 min. Scanning started 30 s before a bolus injection of ~370 MBq of [11C]Flumazenil intravenously over 30 s into an antecubital fossa vein. A metabolite-corrected arterial plasma input function and spectral analysis (Cunningham and Jones, 1993) were used to derive parametric images of volume of distribution (VD) for the ligand.

Statistical analysis

Statistical parametric mapping (SPM99, Wellcome Department of Imaging Neuroscience) was used to make group comparisons of the normalized and smoothed VD images (Friston et al., 1991). A t test was applied at the voxel level to the VD images first to compare all patient groups separately with controls, and a covariate model was used to look at the variation in binding of [11C]Flumazenil with functional (ALSFRS-R score) and clinical measures (disease duration and UMN score) within the separate patient groups.

For each analysis, a proportional voxel threshold of 0.8 (default setting), and proportional scaling of the global VD was used. Those clusters identified with a corrected P < 0.05 or, where there were no significant clusters after correction, those uncorrected clusters whose P values were <0.05, were displayed. Clusters were rendered to a 3D generic brain and, for visualization of internal sections, to the single T1 MRI contained within the SPM software.

Results

Group characteristics

The patient characteristics are summarized in Table 1.

sALS

Twenty-four sALS patients (19 male, five female; mean age 57 years, range 40–72, SD 7), were studied. Three were diagnosed with PMA at the time of investigation. Three patients had bulbar onset of symptoms. The mean
disease duration was 27 months (range 9–80, SD 19). The mean ALSFRS-R score was 38 (range 27–43, SD 5). The mean UMN score was 9 (range 0–15, SD 5).

**homD90A**

Ten homD90A ALS patients (three male, seven female; mean age 53 years, range 36–70, SD 12) were studied. All had limb onset of disease. The mean duration of disease was 68 months (range 9–162, SD 59). The mean ALSFRS-R score was 33 (range 22–44, SD 8). The mean UMN score was 8 (range 3–12, SD 3).

**psD90A**

Two females, aged 44 and 63 years, homozygous for the D90A SOD1 gene mutation, but without symptoms or signs, were also studied. Both are siblings of homD90A cases also included in this study.

**Controls**

Twenty-four healthy controls of similar age (15 male, nine female, mean age 53 years, range 25–68, SD 9) were studied for comparisons.

**PET results**

Significant alterations in cortical [11C]flumazenil binding were noted as follows:

**sALS versus controls**

Decreases in the binding of [11C]flumazenil in the sALS group, compared with controls, were seen bilaterally within the premotor regions, motor cortex (including the medial hemispheric surface) and in posterior motor association areas (Fig. 1).

**HomD90A SOD1 ALS versus controls**

The pattern seen in the homD90A group of ALS patients, compared with controls, differed from the sALS group. Decreases in [11C]flumazenil binding were found in the frontal lobe of the dominant hemisphere, particularly the left fronto-temporal junction, and anterior cingulate gyrus on the medial surface (Fig. 2).

The results from both patient group analyses were not confounded when age was used as a covariate in the model.

**Pre-symptomatic homD90A SOD1 versus controls**

A small area of reduced [11C]flumazenil binding at the left fronto-temporal junction, compared with controls, was seen in the two psD90A subjects. This did not survive cluster correction, but was similar to the area of main decrease seen in the affected homD90A patients, compared with controls. This may therefore represent cortical changes occurring prior to the onset of symptoms in the at-risk subjects (the ‘clinical horizon’) (Fig. 3).

**Clinical correlations in sALS**

Within the sALS group, disease duration did not correlate with decreased [11C]flumazenil binding in any cortical regions. The ALSFRS-R score correlated positively with decreased [11C]flumazenil binding in small clusters located in motor and premotor cortical areas, but these correlations did not survive cluster correction (Fig. 4A). The UMN score,

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Site of disease onset</th>
<th>Disease duration (months)</th>
<th>ALSFRS-R</th>
<th>UMN score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24</td>
<td>15 M : 9 F</td>
<td>53 ± 9</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>sALS</td>
<td>24</td>
<td>19 M : 5 F</td>
<td>57 ± 7</td>
<td>3 BO, 21 LO</td>
<td>27 ± 19</td>
<td>38 ± 5</td>
<td>9 ± 5</td>
</tr>
<tr>
<td>homD90A</td>
<td>10</td>
<td>3 M : 7 F</td>
<td>53 ± 12</td>
<td>10 LO</td>
<td>68 ± 59</td>
<td>33 ± 8</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>psD90A</td>
<td>2</td>
<td>2 F</td>
<td>44 and 63</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

sALS = sporadic amyotrophic lateral sclerosis; homD90A = homozygous D90A SOD1 ALS; psD90A = ‘pre-symptomatic’ unaffected homozygous D90A SOD1 ALS; M = male; F = female; NA = not applicable; BO = bulbar onset; LO = limb onset.
however, correlated negatively with widespread and marked cortical decreases in $[{}^{11}\text{C}]$flumazenil binding over the dominant hemisphere, which remained significant after cluster correction (Fig. 4B).

Clinical correlations in homD90A ALS

In contrast to the sALS patients, there were significant clusters of reduced $[{}^{11}\text{C}]$flumazenil binding that correlated with disease duration in the homD90A ALS patient group (Fig. 5A). These were found in the region of the left fronto-temporal junction, similar to those seen in the comparisons of both affected and psD90A groups versus controls. Furthermore, there were widespread clusters of reduced $[{}^{11}\text{C}]$flumazenil binding in the frontal lobes bilaterally where decreased $[{}^{11}\text{C}]$flumazenil binding correlated with the ALSFRS-R score (Fig. 5B), but not with the UMN score (Fig. 5C).

Discussion

In this $[{}^{11}\text{C}]$flumazenil PET study, we have shown that subjects who have a specific mutation of the $\text{SOD1}$ gene, and who develop a characteristic and slowly progressive ALS phenotype, show a distinctive and less extensive pattern of decreased $[{}^{11}\text{C}]$flumazenil binding than subjects with sALS. We have also confirmed that $[{}^{11}\text{C}]$flumazenil PET reveals both motor and extra-motor cortical involvement in sALS. We have found that cortical $[{}^{11}\text{C}]$flumazenil binding reductions correlate most strongly with upper motor neuron signs in sALS patients, but with functional status (ALSFRS-R) and disease duration in the homD90A group of ALS patients.

While we cannot be certain whether the decreases we observe are due to neuronal loss or dysfunction, or whether they represent a specific failure of GABA-ergic inhibitory function, the two concepts are not mutually exclusive.

A distinctive pattern of selective cerebral involvement in homD90A

Decreased cortical binding of $[{}^{11}\text{C}]$flumazenil in the sALS group was predominantly in premotor, motor and motor
association regions, in keeping with other pathological (Smith, 1960; Petri et al., 2003; Maekawa et al., 2004) and imaging (Kew et al., 1993; Pioro et al., 1994; Lloyd et al., 2000; Ellis et al., 2001) studies. The changes in the sALS group were less widespread than in a previous study using \(^{[11C]}\)flumazenil PET (Lloyd et al., 2000), which may reflect greater heterogeneity and smaller group size in the former study. In the present study, we have shown a different pattern of cortical changes in \(^{[11C]}\)flumazenil binding in sALS compared with homD90A ALS patients of similar disability.

D90A patients worldwide have been shown to share a common founder some 18,000 years ago. To explain this paradox, the existence of a linked disease-modifying factor has been postulated, although the D90A SOD1 mutation may simply be less toxic than others (Andersen et al., 1996; Al-Chalabi et al., 1998; Parton et al., 2002). The distribution of decreased \(^{[11C]}\)flumazenil binding in the homD90A group is less extensive and anatomically distinct from the sALS group, when both groups are compared with healthy controls of similar age. This suggests that the unique genotype and phenotype of homD90A ALS may determine differences in the pattern of cortical vulnerability compared with sALS cases. The distinctive distribution of cortical dysfunction demonstrated by \(^{[11C]}\)flumazenil PET in the homD90A group, which spared the primary motor regions, is the more remarkable given the slightly lower mean ALSFRS-R score (reflecting greater disability) in this group. It is possible that the UMN pathology in the homD90A cases involves less cortical neuronal loss and relatively greater ‘dying back’ change, whereas cortical neuronal loss or dysfunction may be more marked in sALS. To date, no quantitative studies of cortical neuronal loss or axonal damage in the corticospinal tracts have been carried out in the homD90A cases.

Neurophysiological studies have provided evidence that intracortical inhibitory circuits may be selectively preserved in homD90A patients (Weber et al., 2000; Turner et al., 2005), whereas in sALS neurophysiological studies point to increased cortical excitability (Yokota et al., 1996; Ziemann et al., 1997; Hanajima and Ugawa, 1998). The latter may reflect loss or dysfunction of inhibitory GABA-ergic neurons also seen pathologically (Nihei et al., 1992; Maekawa et al., 2004). It is possible that these changes may be related to differential sparing of intra-cortical inhibitory circuits in the homD90A cases compared with sALS.

It is established that up to 40% of sALS patients without frank dementia have deficits in executive function such as verbal fluency (Massman et al., 1996; Abrahams et al., 2000; Lomen-Hoerth et al., 2003), and that a spectrum of fronto-temporal involvement exists (Kew and Leigh, 1992; Lomen-Hoerth et al., 2002). Cognitive abnormalities in ALS, particularly those involving executive function, have been closely linked to frontal lobe dysfunction seen during imaging studies, particularly in the dorso-lateral frontal region (Ludolph et al., 1992; Kew et al., 1993b; Abrahams et al., 1996). To date, there are no published neuropsychological studies within the homD90A patient group, but the predominantly frontal lobe location for the \(^{[11C]}\)flumazenil binding decreases seen in this study would lead us to predict that this group of patients may perform particularly poorly in neuropsychological tests of executive and/or inhibitory function, in excess of the range of deficits seen in many sporadic patients.
Pre-symptomatic cortical changes?
The improved detection of disease in ‘at risk’ subjects would allow current and future disease-modifying therapies to be administered at the earliest opportunity. There is other evidence of pre-symptomatic abnormalities in ‘at risk’ SOD1 gene mutation carriers. A longitudinal neurophysiological study in humans demonstrated a dramatic loss in motor unit number estimates in two carriers of SOD1 gene mutations just prior to the development of paresis (Aggarwal and Nicholson, 2001), and studies in the G93A SOD1 transgenic mouse model of ALS have demonstrated hyperexcitability lower motor neurons (LMNs) in clinically pre-symptomatic animals (Kuo et al., 2004). This study of only two subjects cannot provide unequivocal evidence that cortical changes may be detectable in homD90A subjects who have no symptoms or signs clinically, and further studies are warranted.

Measuring the ‘burden’ of cortical disease in ALS
A surrogate marker of disease activity, as might be used in the monitoring of therapeutic trials, remains elusive in ALS. Quantifying the degree of UMN involvement more accurately is also important, not least because the definite clinical diagnosis of ALS depends on the presence of UMN signs with LMN signs (Brooks et al., 2000). It is known that apparently LMN-only forms of ALS show evidence of UMN degeneration pathologically (Ince et al., 2003). Neurophysiological techniques have shown promise in the detection of UMN involvement (Schulte-Mattler et al., 1999; Triggs et al., 1999).

Disease duration is subjective when onset is taken as the appearance of weakness, and the sALS group by its nature is heterogeneous. The fact that significant correlations with disease duration were seen in the homD90A ALS group, and that these resembled the overall pattern of reduction seen in both affected and pre-symptomatic subjects when compared with controls, may reflect the greater range of disease duration and the smaller sample size. However, it might also reflect the fact that cortical involvement is more closely related to disease duration in this form of the disease.

Little positive correlation was found between reduced [11C]flumazenil binding and the ALSFRS-R scores in the sALS group, whereas there were very marked changes seen in the homD90A patients over the frontal lobes. It is possible that reduced binding is not directly associated with overt functional changes in sALS patients. In the homD90A ALS group, however, the marked frontal localization of the correlation between [11C]flumazenil binding reductions and ALSFRS-R scores would lead us to predict that this functional measure might correlate well with neuropsychological tests of frontal lobe dysfunction.

The 16-point scale of pathological UMN signs, although not validated in the context of a clinical trial, was used in a [11C](R)PK11195 PET study of microglial activation in ALS in vivo, and increased UMN involvement correlated extremely closely with microglial activation in the motor cortex (and to a similar extent within the thalamus) (Turner et al., 2004) (see also Ellis et al., 1999). This UMN scale lends itself more easily to SPM analysis, and may be less subject to co-variation effects caused by clinical heterogeneity within sporadic cases, compared with the ALSFRS-R.

Within the present study, the UMN score appears to correlate well with the extent of cortical disease in sALS, though with the present tracer this seems to be mainly in the dominant hemisphere. Nearly all of the patients were right-handed; there was no predominant laterality to their limb signs. No systematic information exists on asymmetry of cerebral involvement in ALS, but progressive aphasia occurs in ALS, is probably under-recognized and may have characteristic features with relatively selective impairment for processing verbs compared with nouns (see Bak and Hodges, 2004). Further investigation of asymmetries in brain function in ALS are warranted.

Despite this uncertainty, we suggest that this scale is a useful measure of cortical involvement in sporadic ALS for use in imaging studies more widely. In contrast, the weak correlation between [11C]flumazenil binding reduction and the UMN score in the homD90A group (who had a similar range of scores to the sporadic group) lends further support to the idea that UMN pathology in the homD90A cases may involve less cortical neuronal loss and relatively greater corticospinal tract axonopathy. In this context, it is of interest that, following cortical magnetic stimulation of the motor cortex, the very first detectable changes in homD90A cases is a prolongation of corticospinal latency, while amplitude and electromyographic changes appear later (Andersen et al., 1996). As yet there are no reports of the neuropathological changes in homD90A ALS cases to support or refute this notion.

Conclusions
[11C]Flumazenil PET reveals significant differences in the distribution of cerebral involvement in patients with ALS homozygous for the D90A SOD1 gene mutation compared with patients with sporadic disease. This suggests that the nature of selective neuronal vulnerability may differ in the two groups. The more extensive decreases in [11C]flumazenil binding in sALS may reflect more severely disrupted GABA-ergic neurotransmission in these cases, but further work is needed to test this hypothesis directly. Finally, measures of UMN involvement correlate far more with the extent of changes in [11C]flumazenil binding than functional measures of disability in sporadic ALS.

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References
Yokota T, Yoshino A, Inaba A, Saito Y. Double cortical stimulation in Flumazenil PET in sporadic and homD90A ALS 1329...