J Neural Transm (2008) 115: 59–66
DOI 10.1007/s00702-007-0800-y
Printed in The Netherlands

Journal of Neural Transmission

Glycolitic enzymes are targets of oxidation in aged human frontal cortex and oxidative damage of these proteins is increased in progressive supranuclear palsy

A. Martínez, E. Dalfó, G. Muntané, I. Ferrer

Institut de Neuropatologia, Servei Anatomia Patològica, IDIBELL-Hospital Universitari de Bellvitge, Universitat de Barcelona, Hospitalet de Llobregat, Barcelona, Spain

Received 13 February 2007; Accepted 28 July 2007; Published online 21 August 2007

Summary. Progressive supranuclear palsy (PSP) is a neurodegenerative disorder pathologically characterized by neuronal loss and gliosis mainly in specific subcortical nuclei, but also in the cerebral cortex. In addition to neuron loss, hyperphosphorylated tau deposition is found in neurons, astrocytes and coiled bodies. Limited studies have shown that certain oxidative products are increased in the PSP brain. The present study examines oxidative damage in the frontal cortex in 7 PSP compared with 8 age-matched controls. Western blotting of the frontal cortex showed increased 4-hydroxy-2-nonenal (HNE)-immunoreactive bands between 40 and 50 kDa in PSP cases. Bi-dimensional gel electrophoresis and Western blotting, together with mass spectometry, were used to identify HNE-modified proteins. Oxidized phosphoglycerate kinase 1 (PGK-1) and fructose bisphosphate aldolase A (aldolase A) were identified in all cases and 4 of 7 PSP cases, respectively. In contrast, PGK-1 and aldolase A were oxidized in 3 of 8 controls. Immunohistochemistry revealed the localization of aldolase A in neurons and astrocytes, and PGK-1 mainly in astrocytes. These findings show that PGK-1 and aldolase A are targets of oxidation in the frontal cortex in the aged human cerebral cortex and that oxidative damage of these proteins is markedly increased in the frontal cortex in PSP.

Keywords: Progressive supranuclear palsy; oxidative stress; phosphoglycerate kinase 1; fructose bisphosphate aldolase A; astrocytes

Introduction

Progressive supranuclear palsy (PSP) is a degenerative disease manifested with rigidity, loss of balance and unsteady gait, frequent falls, bradykinesia, abnormal eye movements, pseudobulbar palsy, mood and personality disorders, and dementia (Morris et al. 2002; Litvan 2004; Rampello et al. 2005; Scaravilli et al. 2005). The disease is pathologically characterized by atrophy of the midbrain, including superior colliculi, de-pigmentation of the substantia nigra and locus ceruleus, and atrophy of the pallidum, subthalamic nucleus, thalamus, and frontal cortex. Neurofibrillary tangles (NFTs) and neurons with pre-tangles, as well as neuropil threads, are typically encountered in the striatum, globus pallidus, subthalamic nucleus, nucleus basalis of Meynert, several nuclei of the midbrain, pons and dentate nuclei, together with variable NFTs and pre-tangles in the hippocampus, para-hippocampus and isocortex. In addition, tufted astrocytes and coiled bodies in oligodendrocytes are markers of glial involvement in PSP (Litvan et al. 1996; Ikeda et al. 1998; Komori 1999; Dickson 2001; Haw and Agid 2001; Tawana and Ramsden 2001). Hyper-phosphorylated tau, predominantly composed of 4R isoforms, is accumulated in neurons, astrocytes and oligodendrocytes in PSP, and this biochemical hallmark characterizes PSP as a tauopathy (Spillantini and Goedert 1998; Buee et al. 2000; Delacourte and Buee 2000; Lee et al. 2001; Puig et al. 2005).

Oxidative stress has been proposed as playing an important role in the pathogenesis of PSP (Litvan 2004; Rampello et al. 2005). Early studies disclosed high levels of malondialdehyde (MDA), a well-established marker of lipid peroxidation, in the subthalamic nucleus in PSP (Albers et al. 1999). Later, increased MDA levels, and increased levels of 4-hydoxinonenal (HNE), another marker of lipid peroxidation, were reported in the frontal cortex and midbrain tissues, respectively, in PSP cases (Albers

Correspondence: Isidre Ferrer, Institut de Neuropatologia, Servei Anatomia Patològica, IDIBELL-Hospital Universitari de Bellvitge, carrer Feixa Llarga sn, Hospitalet de Llobregat, 08907 Barcelona, Spain
e-mail: 8082ifa@comb.es

et al. 2000; Odetti et al. 2000). Increased levels of the antioxidant superoxide dismutase 1 (SOD1) were found in several brain regions, whereas increased levels of SOD2 were only encountered in the subthalamic nucleus in PSP cases (Cantuti-Castelvetri et al. 2002).

The present study is intended to increase our understanding of oxidative damage and to identify possible targets of oxidation in the frontal cortex in PSP. For this purpose mono- and bi-dimensional gel electrophoresis and Western blotting, followed by in-gel digestion of selected spots and mass spectrometry, were used in 7 cases of PSP and 8 age-matched controls. HNE adducts occurred in phosphoglycerate kinase 1 (PGK 1) and fructose bisphosphate aldolase A in aged human cerebral cortex, and were increased in PSP cases.

Materials and methods

Tissue samples

Brain tissue was obtained as a result of a generous donation for research to the University of Barcelona and Institute of Neuropathology Brain Banks, and following strict criteria of full disclosure and approval designed by the ethics committees of the University of Barcelona and the Hospital Universitari de Bellvitge. Seven sporadic PSP cases were selected for this study. In addition to typical clinical data including parkinsonism, frequent falls and vertical palsy of the gaze, all of them have language deficits and moderate frontosubcortical dysfunction accompanied by grasping and frontal reflexes, and emotional lability. Frontosubcortical dysfunction was neuropsychologically analyzed and characterized by altered attention, preserved orientation, impaired verbal, visual, recent and remote memory, mild or moderate loss of constructive and symbolic praxis, preservation of gnosias, and moderate impairment of calculation. Dependence on others for daily activities and moderate instrumental incapacity (as tested with the RDRS, and Lawton and Brody tests) occurred in all cases. Cortical atrophy, as revealed with CT and MRI, and/or reduced perfusion predominating in the frontal lobes, as revealed with SPECT, were recorded in every case. The duration of the disease was between 4 and 7 years, and the post-mortem delay between death and processing between 4 and 13 h. At autopsy, half of each brain was fixed in 10% formalin, while the other half was cut in coronal sections 1 cm thick, frozen on dry ice and stored at −80°C until use. In addition, samples of the frontal cortex (area 8) were fixed in buffered 4% paraformaldehyde for 48 h, then cryoprotected in 30% saccharose, and frozen at −80°C. Clinical and neuropathological details of these cases are summarized in Table 1. Frozen material was stored for 6–12 months at −80°C, depending on the case. This interval did not have an apparent influence on tissue preservation for biochemical studies (unpublished observations). In addition, eight aged-matched controls were processed in parallel. No neurological signs and symptoms, evidence of metabolic or infectious disease were observed during life. The post-mortem delay was between 2 and 18 h. As in PSP cases, the pH of the brain tissue was between 6.8 and 7. The neuropathological study did not reveal abnormalities following the examination of the same regions and using the same battery of techniques as those utilized in diseased cases.

Table 1. *Summary of the cases studied in the present series*

Case	Age	Gender	Post-mortem delay	Diagnosis
1	71	M	4	PSP
2	65	F	9	PSP
3	69	M	9	PSP
4	67	M	9	PSP
5	65	F	6	PSP
6	68	F	13	PSP
7	72	F	4	PSP
8	58	M	4	C
9	79	M	7	C
10	85	M	6	C
11	73	F	5	C
12	66	M	2	C
13	70	M	13	C
14	65	M	4	C
15	18	F	18	C

Age in years; Post-mortem delay in hours; *diagnosis* neurological and neuropathological diagnosis, *PSP* progressive supranuclear palsy; *C* no neurological and neuropathological lesions.

Gel electrophoresis and western blotting

Brain samples (0.1 g) of the frontal cortex from PSP and control cases were homogenized in 1 ml of lysis buffer (10 mM Tris-HCl pH 7.4, 100 mM NaCl, 10 mM EDTA, 0.5% sodium deoxycholate, 0.5% NP-40, 1 mM sodium orthovanadate and Complete protease inhibitor cocktail (Roche Molecular Systems, Barcelona, Spain) and centrifuged at 5000 rpm (2600 g) for 10 min at 4°C. The pellets were discarded and protein concentrations of the supernatants were determined by BCA (bicinchoninic acid) method with bovine serum albumin (BSA) (Sigma, Barcelona, Spain) as a standard.

Samples containing 30 µg of protein were loaded onto 10% acrylamide gels and proteins were separated by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electroforesis) and then transferred to nitrocellulose membranes (200 mA/gel for 90 min). After transfer, the membranes were treated with $NaBH_4$; they were washed for 5 min in Tris-buffer saline (TBS) three times and incubated for 30 min with $NaBH_4$ 10 mM. Then the membranes were blocked with 5% skimmed milk in TBS-T buffer (100 mM Tris-buffered saline, 140 mM NaCl and 0.1% Tween 20, pH 7.4) for 1 h at room temperature and incubated with the primary antibody in TBS-T-3% bovine serum albumin (BSA) at 4°C overnight. The membranes were incubated with rabbit polyclonal anti-HNE antibody (Calbiochem, Barcelona, Spain). Later, the membranes were washed for five minutes in TBS-T three times and then incubated for 45 min at room temperature with the corresponding secondary antibody labeled with horseradish peroxidase (Dako, Barcelona, Spain) at a dilution of 1:1000, and then washed in TBS-T for 30 min. Immunoreactivity was detected with the chemiluminescence ECL method (Amersham, Barcelona, Spain). The monoclonal antibody to β-actin (Sigma, Madrid, Spain) was used at a dilution of 1:10,000 as a control of protein loading.

Densitometry and statistical processing of data

Protein expression levels were determined by densitometry of the bands using Total laboratory v2.01 software. This software detects the bands obtained by Western blot and gives individual values which are dependent on the light quantification of the corresponding band. Measurements are expressed as arbitrary units. The results were normalized for β-actin. The numerical data obtained from PSP and the corresponding controls were statistically analyzed using the Student *t*-test. Asterisk indicates p values < 0.05.

2D gel electrophoresis

In the first dimension electrophoresis, samples were applied onto 7 cm pH 3–10 nonlinear gradient ReadyStrip IPG strips (Bio-Rad) for isoelectric

focusing (IEF). The strips were actively re-hydrated at 50 V for 16 h and the proteins were focused at 300 V for 2 h, after which the voltage was gradually increased to 500 V for 2 h. Focusing was continued at 1000 V for 2 h and at 8000 V for 18 h. After focusing, the IEF strips were stored at −80°C until the second dimension electrophoresis was performed.

For the second dimension electrophoresis, IPG strips were equilibrated for 10 min in 50 mM Tris-HCl (pH 6.8) containing 6 M urea, 1% (wt/v) SDS (sodium dodecyl sulfate), 30% (v/v) glycerol, and 2% dithiotreitol, and then re-equilibrated for 10 min in the same buffer containing 2.5% iodacetamide. The strips were placed on 10% polyacrilamide gels, together with standards (Fermentas, Ontario, Canada) at 20 mA/gel 2. For the second dimension, two gels from the same case were run in parallel. One replicate was stained with silver and the other replicate was transferred to a nitrocellulose membrane (200 mA/gel for 90 min). For gel staining, a mass spectometry-modified silver staining method (Amersham) was used as described by the manufacturer. Transferred nitrocellulose membranes were processed for blotting. After incubation with 5% skimmed milk in TBS-T buffer for 1 h at room temperature, nitrocellulose membranes were incubated with anti-HNE (Calbiochem), used at dilution of 1, 1000. Membranes were also incubated with rabbit anti-phosphoglycerate kinase 1 (Abgent, San Diego, CA, USA) or goat anti-fructose bisphosphate aldolase A (Novus Biologicals, Littleton, USA) antibodies used at dilutions of 1:150 and 1:10,000, respectively. The immunoreaction was visualized as previously.

In-gel digestion

Proteins were in-gel digested with trypsin (Promega, Barcelona, Spain) in the automatic Investigator ProGest robot of Genomic Solutions. Briefly, excised gel spots were washed sequentially with ammonium bicarbonate buffer and acetonitrile. Proteins were reduced with 10 mM DTT (dithiothreitol) solution for 30 min and alkylated with 100 mM solution of iodine acetamide. After sequential washings with buffer and acetonitrile, proteins were digested overnight at 37°C with trypsin 0.27 nM. Tryptic peptides were extracted from the gel matrix with 10% formic acid and acetonitrile. The extracts were pooled and dried in a vacuum centrifuge.

Acquisition of MS and MS/MS spectra

Proteins manually excised from the 2D gels were digested and analyzed by CapLC-nano-ESI-MS-MS mass spectrometry. The tryptic digested peptide samples were analyzed using on-line liquid chromatography (CapLC, Micromass-Waters, Manchester, UK) coupled to tandem mass spectrometry (Q-TOF Global, Micromass-Waters). Samples were resuspended in 12 µl of 10% formic acid solution and 4 µl was injected for chromatographic separation into a reverse-phase capillary C_{18} column (75 µm of internal diameter and 15 cm in length, PepMap column, LC Packings, Amsterdam, The Netherlands). The eluted peptides were ionized via coated nano-ES needles (PicoTip™, New Objective, Woburn, USA). A capillary voltage of 1800–2200 V was applied together with a cone voltage of 80 V. The collision in the CID (collision-induced dissociation) was 25–35 eV and argon was employed as the collision gas. Data were generated in PKL file format and submitted for database searching in MASCOT server (Matrix Science, USA) using the NCBI database with the following parameters: trypsin enzyme, 1 missed cleavage, carbamidomethyl (C) as fixed modification and oxized (M) as variable modification, and mass tolerance of 150–250 ppm.

Probability-based MOWSE score was used to determine the level of confidence in the identification of specific isoforms from the mass spectra. This probability equals $10^{(-\text{Mowse score}/10)}$. Mowse scores greater than 50 were considered to be of high confidence of identification.

Immunohistochemistry to phosphoglycerate kinase 1 and bisphosphate aldolase A

Cryostat sections 14 microns thick of cryoprotected frontal cortex were processed free-floating with the EnVision + system peroxidase procedure (Dako, Barcelona, Spain) in every case. Control samples were

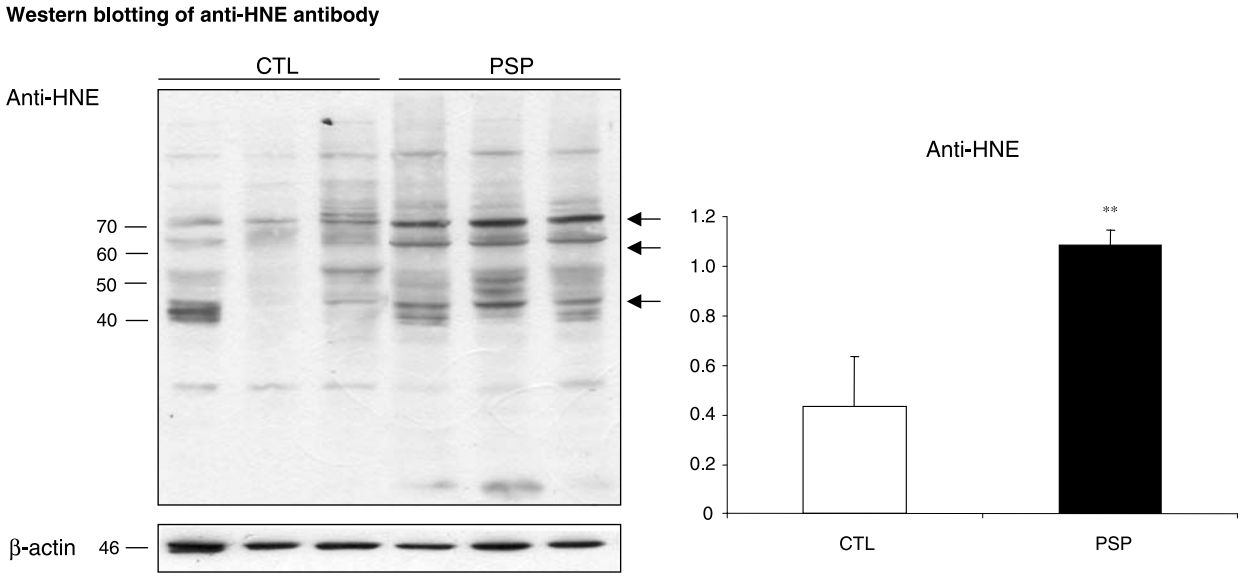

Fig. 1. Mono-dimensional gel electrophoresis and Western blotting of frontal cortex homogenates blotted for HNE show increased immunoreactivity of bands of 60 and 70 kDa and several bands between 40 and 50 kDa in progressive supranuclear palsy (*PSP*) when compared with controls (*CTL*). Quantitative analysis of HNE-immunoreactive bands, corrected for β-actin, expressed as mean values ± SD, reveal a significant increase in the intensity of all the bands between 40 and 70 kDa in PSP cases compared with CTL. Student T test: * $p < 0.05$. Cases represented in the figure correspond to 8, 15 and 10 (controls), and 1, 2 and 4 (PSP)

processed in parallel. The rabbit anti-phosphoglycerate kinase 1 antibody (Abgent, San Diego, CA, USA) was used at a dilution of 1:150. The goat anti-fructose bisphosphate aldolase A antibody (Novus Biologicals, Littleton, USA) was used at a dilution of 1:5000. The sections were incubated with EnVision for 1 h at room temperature. The peroxidase reaction was visualized with NH_4NiSO_4 (0.05 M) in phosphate buffer (0.1 M), 0.05% diaminobenzidine, NH_4Cl and 0.01% hydrogen peroxide (dark blue precipitate). Some sections were incubated without the primary antibody. No immunoreactivity was found in these samples.

Results

Mono-dimensional gel electrophoresis and western blotting to lipoxidized products

Western blot analysis of frontal cortex homogenates incubated with the anti-HNE antibody revealed increased intensity of bands of about 70, 60, and between 40 and 50 kDa in PSP cases when compared with the majority of controls (Fig. 1). Densitometric studies, including all the cases examined, revealed that these differences were significant ($p < 0.05$).

2D gel electrophoresis, in gel digestion and mass spectomtery

Immunodetection of oxidized proteins was performed using anti-HNE antibody. Two rows of spots located at the level of the 40 and 50 kDa markers were selected for study. Silver-stained spots of gels run in parallel were in-gel digested and processed for MS (Fig. 2). The same spots were identified in every case. The proteins identified were phoshoglycerate kinase 1 (PGK-1) and fructose bisphosphate aldolase A (aldolase A) (Table 2).

Validation

To confirm the identity of these proteins, the same membranes were blotted with anti-HNE and anti-PGK 1 antibod-

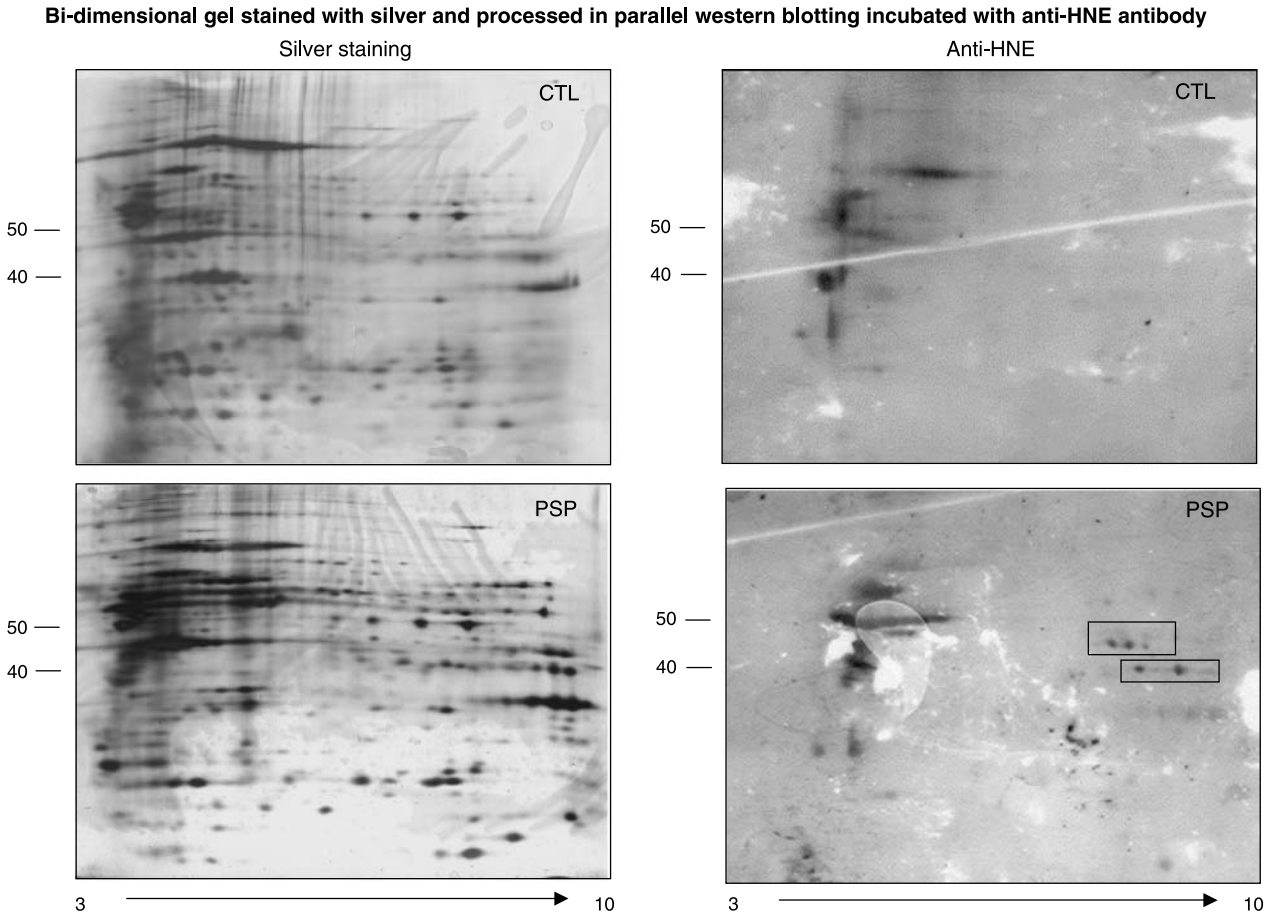

Fig. 2. 2D gel electrophoresis and immunoblotting with anti-HNE in control (CTL, case 15) and PSP (case 1) shows two rows of HNE-immunoreactive spots in PSP and not in CTL (*right panel*) between 40 and 50 kDa. Immunoreactive spots were matched in parallel gels stained with silver (*left panel*), which were in-gel digested, and processed for mass spectometry

Table 2. *Oxidized proteins excised from PSP gels. Spots were obtained from cases 1 (1, 2, 3, 4), 6 (5, 6) and 7 (7)*

	Calculated pI	Nominal mass (Da)	Protein	Score coverage	No. of peptides matched	GI accession
1	8.3	44854	Phospho-glycerate kinase 1	58	5	P00558
2	8.3	44854	Phospho-glycerate kinase 1	54	9	P00558
3	8.39	39720	Fructose-bisphosphate aldolase A	60	4	P04075
4	8.39	39720	Fructose-bisphosphate aldolase A	73	8	P04075
5	8.3	44985	Phospho-glycerate kinase 1	204	6	NP000282
6	8.39	39816	Fructose-bisphosphate aldolase A	171	4	CAA30270
7	8.3	44985	Phospho-glycerate kinase 1	292	6	NP000282

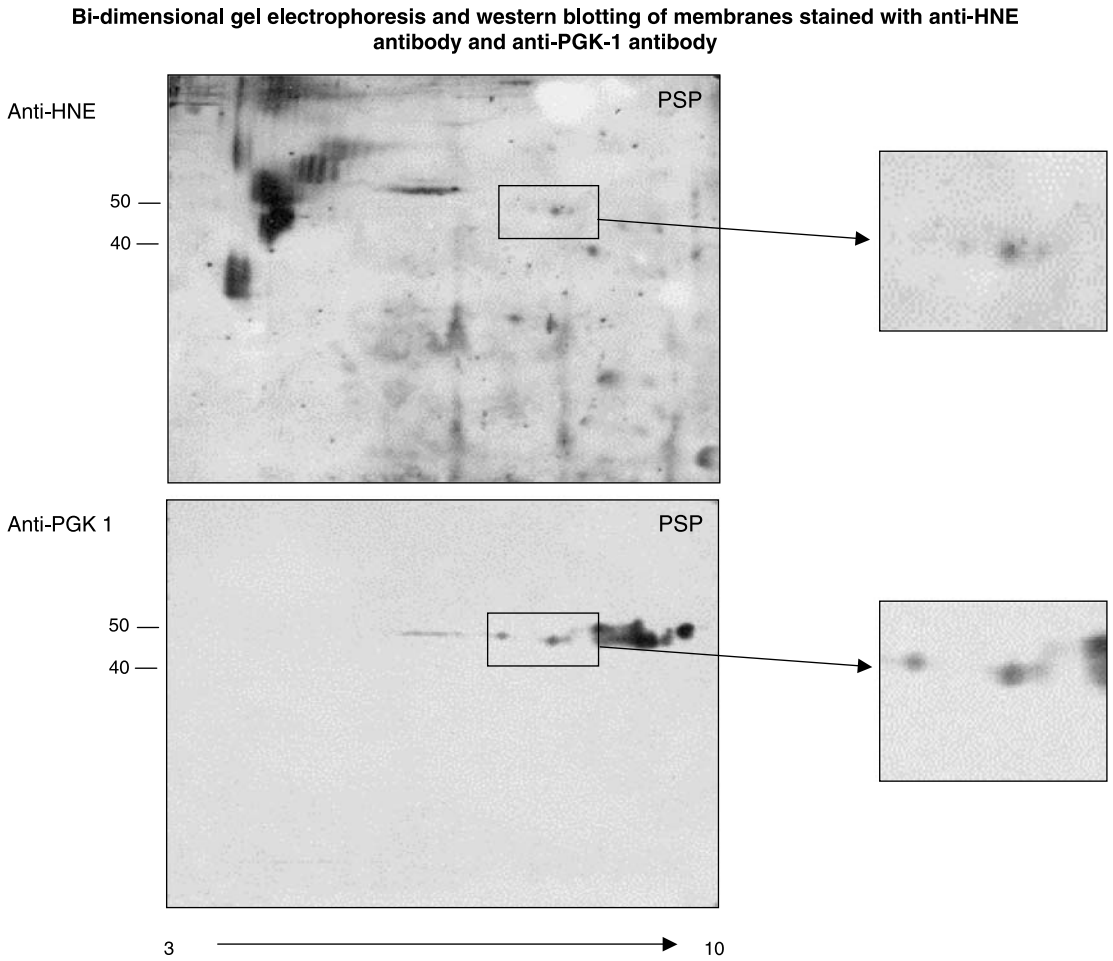

Fig. 3. Bi-dimensional gel electrophoresis and Western blotting of membranes stained with anti-PGK-1 antibody (*upper panel*) and anti-aldolase A (*lower panel*) antibodies in PSP (case 1)

ies. Selected spots were stained with anti-HNE and anti-PGK 1 antibodies (Fig. 3). Similarly, other pairs of membranes were blotted with anti-HNE and anti-aldolase A antibodies, showing similar results (Fig. 3).

The same studies were carried out in every case. Examples of these membranes are shown in Fig. 4. Lipoxidized PGK-1 and aldolase A were present in the 3 of 8 control cases, whereas oxidized PGK-1 occurred in 7 of 7 and oxidized aldolase A in 4 of 7 PSP cases (Table 3).

PGK-1 and aldolase A immunohistochemistry

Aldolase A immunoreactivity was present in neurons and glial cells in control and in diseased brains (Fig. 5A). PGK-1

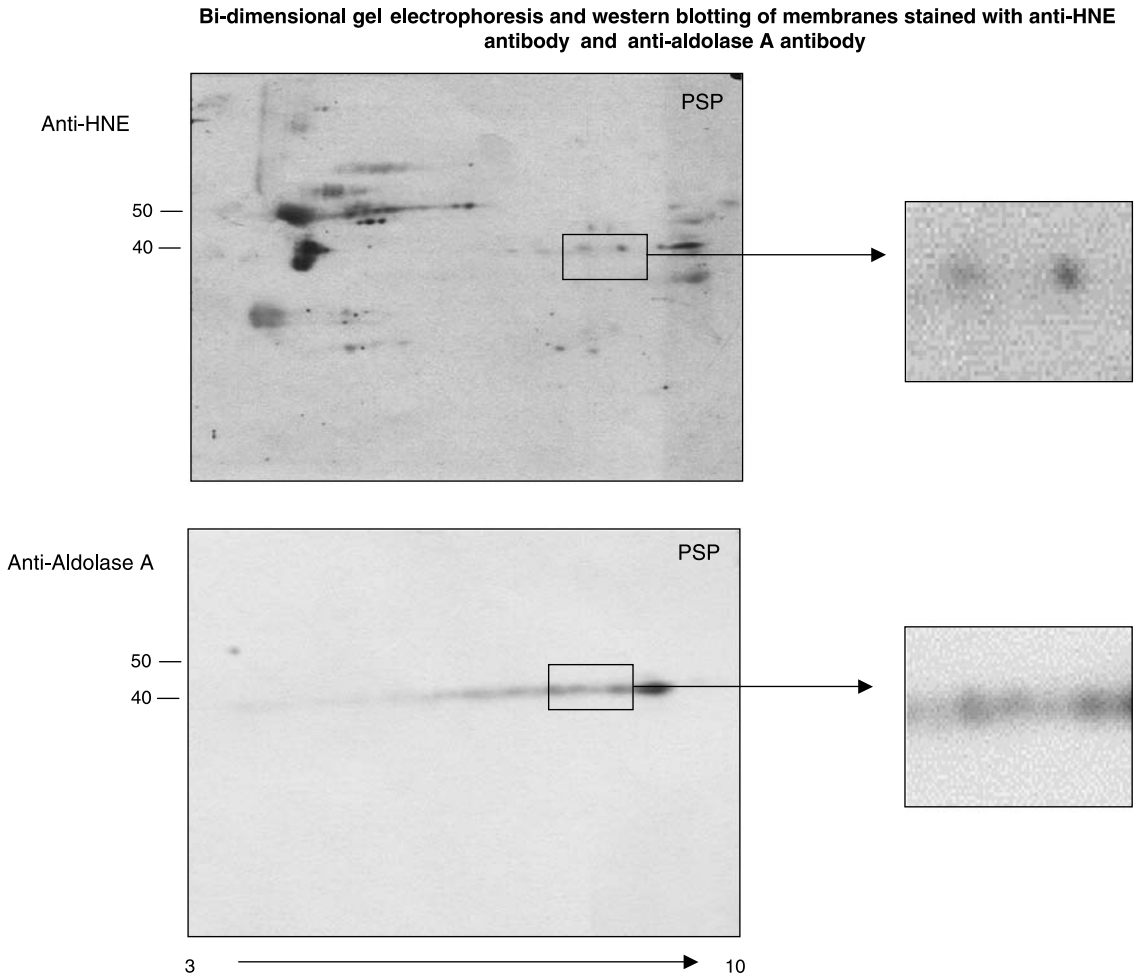

Fig. 4. Bi-dimensional gel electrophoresis and Western blotting of membranes stained with anti-HNE antibody (*left panel*) corresponding to one control (case 14) and one PSP case (case 6) showing parallel rows of positive spots between 40 and 50 kDa in PSP. Parallel membranes of the PSP case immunostained with anti-PGK-1 and fructose-bisphosphate aldolase A antibodies show specific immunostaining of the corresponding rows

Table 3. *Oxidized phospho-glycerate kinase 1 (PGK-1) and fructose-bisphosphate aldolase A (aldolase A) in PSP and control cases*

Case	Diagnosis	Oxidized PGK-1	Oxidized aldolase A
1	PSP	yes	yes
2	PSP	yes	yes
3	PSP	yes	no
4	PSP	yes	yes
5	PSP	yes	no
6	PSP	yes	yes
7	PSP	yes	no
8	C	no	no
9	C	yes	yes
10	C	no	no
11	C	no	no
12	C	yes	yes
13	C	yes	yes
14	C	no	no
15	C	no	no

immunoreactivity was mainly observed in the cytoplasm of astrocytes (Fig. 5B). No differences in the intensity of the immunostaining were found between control and diseased cases in individual cells excepting the increased number of astrocytes in PSP. It is worth mention that these antibodies do not distinguish oxidized from non-oxidized forms of the enzymes.

Discussion

Since previous reports have shown increased lipoxidative damage in the frontal cortex in PSP (Albers et al. 2000), the present study was delineated to gain understanding about targets of lipoxidative damage in the cerebral cortex in PSP brains.

2D gel electrophoresis, Western blotting to HNE and mass spectrometry has permitted the identification of two

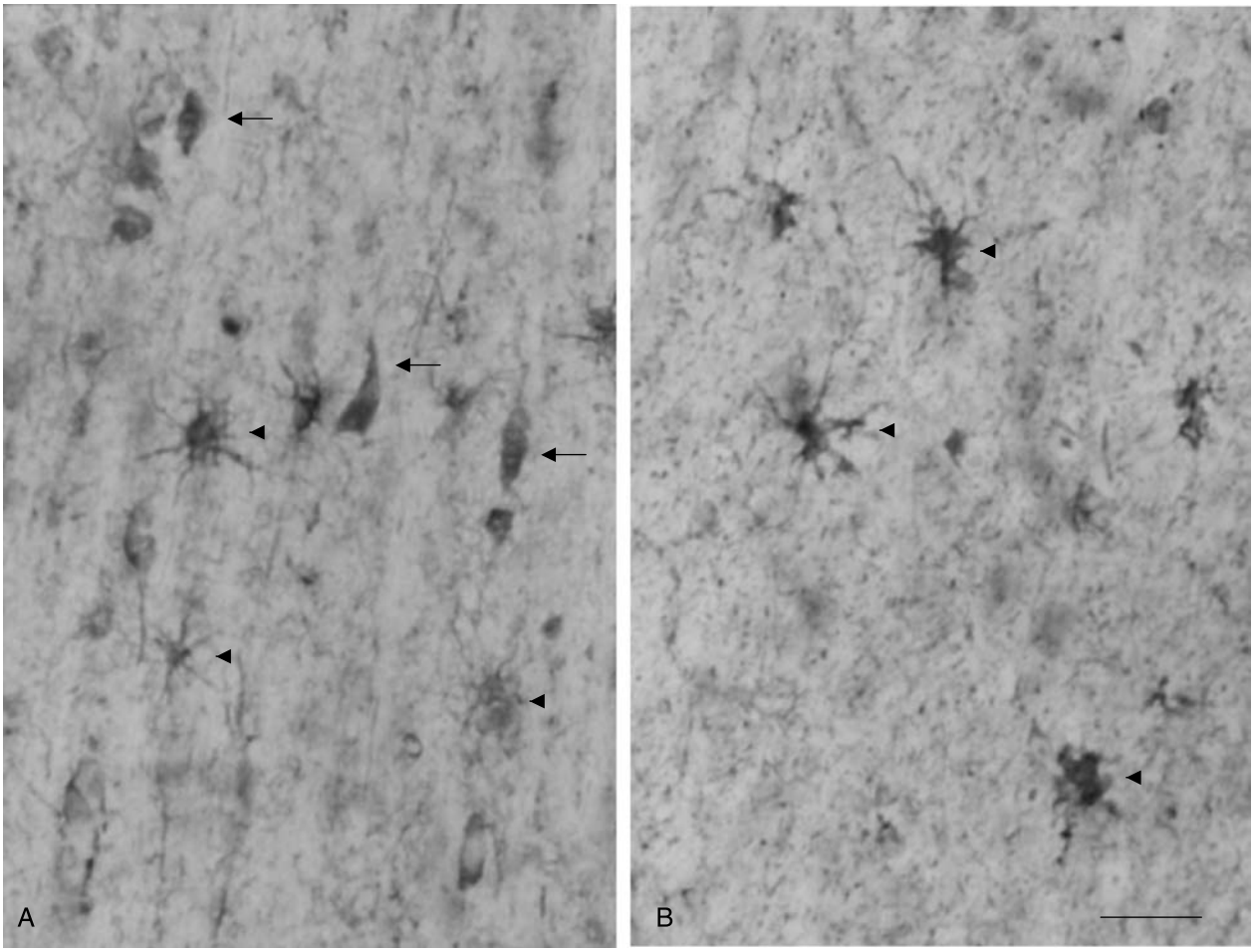

Fig. 5. Immunohistochemistry to aldolase A (**A**) and PGK-1 (**B**) in the PSP frontal cortex (case 1). Aldolase A immunoreactivity is observed in the cytoplasm of neurons (*arrows*) and astrocytes (*arrowheads*). PGK-1 immunoreactivity is mainly found in the cytoplasm of astrocytes (*arrowheads*). Cryostat section processed free-floating with no counterstaining. Bar = 25 μm

proteins that are oxidized in the frontal cortex in PSP cases: fructose bisphosphate aldolase A (aldolase A) and phosphoglycerate kinase 1 (PGK-1). Both enzymes are implicated in glycolysis. Fructose 1,6-bisphosphate aldolase A converts fructose-1,6-bisphosphate into glyceraldehyde-3-phosphate and dihidroxyacetone phosphate. PGK-1 catalyzes the conversion of 1,3-diphosphoglycerate to 3-phosphoglycerate, leading to the production of the first ATP in glycolysis. Loss of function of PGK-1 may impair glucose metabolism and result in the accumulation of glycolytic intermediates.

PGK-1 has been described as being oxidized in the frontal cortex in Alzheimer's disease (AD) (Castegna et al. 2002; Korolainen et al. 2006) and in transgenic Tg2576 mice with Alzheimer plaque pathology (Bigl et al. 2003). Several proteins related to glycogenesis and glycolysis have also been reported to be oxidized in experimental models of AD following injection of amyloid β peptide (1–42) into rat brain (Boyd-Kimball et al. 2005) and in rat primary neural cells following amyloid β_{1-42}-induced oxidative damage (Sultana et al. 2006). Therefore, oxidation of proteins linked with the glycolysis may account for impaired energy metabolism in the AD brain.

No antecedents of HNE-modified glycolytic enzymes have been reported so far in PSP. Yet previous studies using (18F)-2-fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET) have shown a significant alteration of cerebral glucose metabolism in the frontal cortex in PSP cases (Karbe et al. 1992). The present findings suggest that oxidation of aldolase A and PGK-1 may also account for impaired energy metabolism in PSP, and they provide a biochemical substrate for impaired glucose metabolism in PSP. Since aldolase A is present in neurons and astrocytes, and PGK-1 mainly in astrocytes, the present findings support neurons and astrocytes as targets of oxidative damage in PSP.

Although PGK-1 has been found to be oxidized in 7 of 7 cases and aldolase A in 4 of 7 PSP cases, oxidation of these proteins has also been observed in certain control cases. Thus PGK-1 and aldolase A were oxidized in the frontal cortex of 3 of 8 control cases. No apparent clinical and neuropathological differences were noticed between these three cases and the remaining five controls with no evidence of PGK-1 and aldolase A oxidation, excepting the young age in a single case.

In summary, the present study has shown that aldolase A and PGK-1 as targets of lipoxidation in the aged human cerebral cortex, and that increased lipoxidation of aldolase A and, particularly, of PGK-1 occurs in the frontal cortex in PSP. The present findings together with previous observations in AD support the idea that increased oxidation of proteins linked with glycolysis is a common feature in advanced stages of certain neurodegenerative diseases including AD and PSP.

Acknowledgements

This work was funded by grants from the Spanish Ministry of Health, Instituto de Salud Carlos III (PI040184 and PI05/1570), and supported by the European Commission under the Sixth Framework Programme (BrainNet Europe II, LSHM-CT-2004-503039). We thank T. Yohannan for editorial help.

References

Albers DS, Augood SJ, Martin DM, Standaert DG, Vonsattel JP, Beal MF (1999) Evidence for oxidative stress in the subthalamic nucleus in progressive supranuclear palsy. J Neurochem 73: 881–884

Albers DS, Augood SJ, Park LC, Browne SE, Martin DM, Adamson J, Hutton M, Standaert DG, Vonsattel JP, Gibson GE, Beal MF (2000) Frontal lobe dysfunction in progressive supranuclear palsy: evidence for oxidative stress and mitochondrial impairment. J Neurochem 74: 878–881

Bigl ML, Apelt J, Eschrich K, Schliebs R (2003) Cortical glucose metabolism is altered in aged transgenic Tg2576 mice that demonstrate Alzheimer plaque pathology. J Neural Transm 110: 77–94

Boyd-Kimball D, Sultana R, Poon HF, Lynn BC, Casamenti F, Pepeu G, Klein JB, Butterfield DA (2005) Proteomic identification of proteins specifically oxidized by intracerebral injection of amyloid peptide (1–42) into rat brain: implications for Alzheimer's disease. Neuroscience 132: 313–324

Buee L, Bussiere T, Buee-Scherrer V, Delacourte A, Hof PR (2000) Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. Brain Res Rev 33: 95–130

Cantuti-Castelvetri I, Keller-McGandy CE, Albers DS, Beal MF, Vonsattel JP, Standaert DG, Augood SJ (2002) Expression and activity of antioxidants in the brain in progressive supranuclear palsy. Brain Res 930: 170–181

Castegna A, Aksenov M, Thonboonkerd V, Klein JB, Pierce WM, Booze R, Markesbery W, Butterfield DA (2002) Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part II: Dihydropyrimidinase-related protein 2, α-enolase and heat shock cognate 71. J Neurochem 82: 1524–1532

Delacourte A, Buee L (2000) Tau pathology: a marker of neurodegenerative disorders. Curr Opin Neurol 13: 371–376

Dickson DW (2001) Progressive supranuclear palsy and corticobasal degeneration. In: Hof PR, Mobbs ChV (eds) Functional neurobiology of aging. Academic Press, San Diego, pp 155–171

Hauw JJ, Agid Y (2003) Progressive supranuclear palsy (PSP) or Steele-Richardson-Olszewski disease. In: Dickson D (ed) Neurodegeneration: the molecular pathology of dementia and movement disorders. ISN Neuropath Press, Basel, pp 103–114

Ikeda K, Akiyama H, Arai T, Nishimura T (1998) Glial tau pathology in neurodegenerative diseases: their nature and comparison with neuronal tangles. Neurobiol Aging 19: S85–S91

Karbe H, Grond M, Huber M, Herholz K, Kessler J, Heiss WD (1992) Subcortical damage and cortical dysfunction in progressive supranuclear palsy demonstrated by positron emission tomography. J Neurol 239: 98–102

Komori T (1999) *Tau*-positive glial inclusions in progressive supranuclear palsy, corticobasal degeneration and Pick's disease. Brain Pathol 9: 663–679

Korolainen MA, Goldsteins G, Nyman TA, Alafuzoff I, Koistinaho J, Pirttila T (2006) Oxidative modification of proteins in the frontal cortex of Alzheimer's disease brain. Neurobiol Aging 27: 42–53

Lee WM, Goedert M, Trojanowski JQ (2001) Neurodegenerative tauopathies. Annu Rev Neurosci 24: 1121–1159

Litvan I (2004) Update on progressive supranuclear palsy. Curr Neurol Neurosci Rep 4: 296–302

Litvan I, Hauw JJ, Bartko JJ, Lantos PL, Daniel SE, Horoupian DS, McKee A, Dickson D, Bancher C, Tabaton M, Jellinger K, Anderson DW (1996) Validity and reliability of the preliminary NINDS neuropathologic criteria for progressive supranuclear palsy and related disorders. J Neuropathol Exp Neurol 55: 97–105

Morris HR, Gibb G, Katzenschlager R, Wood NW, Hanger DP, Strand C, Lashley T, Daniel SE, Lees AJ, Anderton BH, Revesz T (2002) Pathological, clinical and genetic heterogeneity in progressive supranuclear palsy. Brain 125: 969–975

Odetti P, Garibaldi S, Norese R, Angelini G, Marinelli L, Valentini S, Menini S, Traverso N, Zaccheo D, Siedlak S, Perry G, Smith MA, Tabaton M (2000) Lipoperoxidation is selectively involved in progressive supranuclear palsy. J Neuropathol Exp Neurol 59: 393–397

Puig B, Rey MJ, Ferrer I (2005) Individual and regional variations of phospho-tau species in progressive supranuclear palsy. Acta Neuropathol 110: 261–268

Rampello l, Buttà V, Raffaele R, Vecchio I, Battaglia G, Cormaci G, Alvano A (2005) Progressive supranuclear palsy: a systematic review. Neurobiol Dis 20: 179–186

Scaravilli T, Tolosa E, Ferrer I (2005) Progressive supranuclear palsy and corticobasal degeneration: lumping versus splitting. Mov Disord 20 (Suppl 12): S21–S28

Spillantini MG, Goedert M (1998) Tau protein pathology in neurodegenerative diseases. Trends Neurosci 21: 428-433

Sultana R, Newman SF, Abdul HM, Cai J, Pierce WM, Klein JB, Merchant M, Butterfield DA (2006) Protective effect of D609 against amyloid-beta$_{1-42}$-induced oxidative modifications on neuronal proteins: redox proteomics studies. J Neurosci Res 84: 409–417

Tawana K, Ramsden DB (2001) Progressive supranuclear palsy. Mol Pathol 54: 427–434