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Review Article

Oxidative stress in developmental brain disorders

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Oxidative stress is one of the predisposing factors in adult neurological disorders. We have examined the involvement of oxidative stress in child-onset neurodegenerative disorders, and here we review the findings from our analysis. In cases of Cockayne syndrome, the oxidative products of lipids and proteins were increased in the globus pallidus; however, oxidative nucleotide damage that coincided with reduced copper/zinc superoxide dismutase (Cu/ZnSOD) expression was observed in cases of xeroderma pigmentosum, and these patients also presented increased oxidative stress markers in urine samples. In spinal muscular atrophy, lipid peroxidation in conjunction with oxidative DNA damage was observed in motor neurons. Cases of subacute sclerosing panencephalitis presented oxidative nucleoside damage in cerebral cortical neurons at early disease stages, which were subsequently replaced by lipid peroxidation in glial cells of cerebral white matter. In relation to progressive myoclonic epilepsy, oxidative damage to DNA, proteins, and lipids appeared to coincide with cerebral and cerebellar cortical lesions of neuronal ceroid-lipofuscinosis. Patients with Lafora disease also presented an increase in oxidative stress markers for DNA and/or lipids in the brain and urine. These findings imply involvement of oxidative stress in developmental brain disorders; antioxidant agents could prove to be useful for treating patients with those disorders.

Key words: oxidative stress, progressive myoclonic epilepsy, spinal muscular atrophy, subacute sclerosing panencephalitis, xeroderma pigmentosum.

INTRODUCTION

Free radicals are defined as molecules or molecular fragments with one or more unpaired electrons. Tissue damage caused by oxidative stress, mediated by excessive free radicals, is involved in a diversity of biological phenomena, including aging, carcinogenesis, atherosclerosis, and neurodegeneration.[1] Oxidative stress originates from an imbalance between production of reactive oxygen/nitrogen species and antioxidant capacities of cells and organs. Reactive oxygen species (ROS) include superoxide anion ($O_{2\cdot}$), hydroxyl radicals (.OH), and hydrogen peroxide (H_2O_2), while antioxidants are composed of several vitamins and endogenous enzymes, such as catalase, superoxide dismutase (SOD), and glutathione peroxidase. SOD converts O_2^- into H_2O_2, which is rapidly reduced by catalase and glutathione peroxidase.[2] When the production of ROS exceeds the detoxification of ROS, the balance shifts towards oxidative stress. Oxidative stress to lipids, proteins, and nucleotides results in the accumulation of substrate-specific substances known as oxidative stress markers.[3] Lipid peroxidation can cause disruption of cell membranes, thereby leading to their destruction; early- and late-stage markers for lipid peroxidation include hexanoyl-lysine adduct (HEL), acrolein-lysine adduct (ACR), and 4-hydroxy nonenal (4-HNE).[4,5] Oxidative damage to DNA and RNA produces 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 8-hydroxyguanosine (8-OHG), respectively, which are known markers of oxidative nucleoside damage.[6] Advanced glycation end products (AGE) are considered to be markers of protein damage by glycoxidation; the generation of AGE has been described in aging, atherosclerosis, and in the progression of diabetes mellitus.[7]

ROS are abundantly produced in the brain; neurons consume a large amount of oxygen, neuronal mitochondria generate $O_{2\cdot}$, and the brain readily retains bio-available irons.[1,8] Oxidative stress has been shown to be one of the predisposing factors for neurodegeneration in several adult-onset neurological disorders, such as Alzheimer's

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disease, Parkinson's disease, and amyotrophic lateral sclerosis.[9] In Alzheimer's disease, enhanced lipid peroxidation, as well as increased oxidized proteins and nucleotides, have been observed in cell culture, transgenic animals, postmortem brains, and cerebrospinal fluids from patients.[10] Oxidative stress has also been shown to be involved in dopaminergic neuronal loss in Parkinson's disease.[11] Mutations in the gene for copper/zinc superoxide dismutase (Cu/ZnSOD) can cause familial amyotrophic lateral sclerosis,[12] and patients with sporadic amyotrophic lateral sclerosis revealed an increase in 3-nitrotyrosine (NT), which can be ameliorated by repetitive injections of edaravone, a free radical scavenger.[13] Since oxidative stress has also been discovered in Down syndrome and periventricular leukomalacia,[14,15] we performed immunohistochemical analysis of oxidative products and expression of SOD in autopsy brains of child-onset neurodegenerative disorders. More recently, oxidative stress markers have become available to evaluate levels of oxidative DNA damage and lipid peroxidation in urine, serum, and CSF samples from children with neurological disorders.[16,17] We also started an ELISA analysis for oxidative stress markers using urine and CSF samples from patients. In this study, we review the results of immunohistochemical analyses in autopsy brains and preliminary ELISA findings in urine samples.

MATERIALS AND METHODS

Subjects comprised of five cases each of xeroderma pigmentosum group A (XPA) and Cockayne syndrome (CS),[18,19] seven cases of spinal muscular atrophy (SMA),[20,21] six cases of subacute sclerosing panencephalitis (SSPE),[22] five cases of neuronal ceroid lipofuscinosis (NCL),[23,24] and three cases of Lafora disease (LD). In addition, five controls were included in the study, who were without brain disorders and were 9 to 29 years of age. In serial sections of the frontal and temporal cortex, basal ganglia, thalamus, cerebellar cortex with the dentate nucleus, and/or midbrain, we performed immunohistochemistry using monoclonal antibodies against 8-OHG (1:300, Trevigen, Gaithersburg, MD, US), 8-OHdG, 4-HNE (1:2000, Wako Pure Chemicals industries, Osaka, Japan), AGE (1:2000, Trans Genic Inc., Kumamoto, Japan) and NT (1:500, Upstate Biotechnology, Lake Placid, NY, US), and polyclonal antibodies for Cu/ZnSOD and MnSOD (1:3000 and 1:2000, Stressgen, BC, Canada). To determine the nucleotide species in the 8-OHG and 8-OHdG immunohistochemistry analysis, sections were pretreated with deoxyribonuclease and ribonuclease.[19,22] Five controls did not present any accumulation of 8-OHG, 8-OHdG, 4-HNE, AGE, or NT in the brain regions examined, and presented preserved expressions of SOD, as reported previously.[19] Levels of late-stage markers for lipid peroxidation, HEL and ACR, and 8-OHdG were determined by ELISA (Japan Institute for the Control of Aging, Shizuoka, Japan) of urine samples from four XPA, one XPD,[25] and two genetically confirmed LD[26] patients, in addition to 17 healthy controls (3–81 years old). The patients and/or their parents approved sampling of urine. All analyses were performed in triplicate and blinded. Urinary creatinine (mg/dL) was measured by a standard automated colorimetric assay, and each value was expressed relative to urinary creatinine to adjust for muscle mass.

RESULTS

Xeroderma pigmentosum (XP) and Cockayne's syndrome (CS)

XP, CS, and trichothiodystrophy are rare neurocutaneous disorders caused by inherited disturbance in nucleotide excision repair.[27] Patients demonstrate skin hypersensitivity to sun exposure, in association with neurological abnormalities. Complementation studies have revealed the existence of eight genes in XP (group A–G and an XP-variant) and two in CS (groups A and B). XP (groups A, B, D and G) and CS patients show progressive neurological disorders, including peripheral and acoustic neuropathy, cerebellar ataxia, pyramidal and extrapyramidal motor abnormalities, and mental disturbances.[28] Neuropathologically, both autopsy cases of XPA and CS demonstrated a common occurrence of grumose foamy spheroid bodies (GFSB) in the globus pallidus and substantia nigra,[29] as well as apoptotic neuronal death in the hippocampal and cerebellar lesions.[30] In CS cases, there was significant deposition of NT, AGE, and 4-HNE in the globus pallidus, indicating oxidative stress, compared to the XPA cases (Table 1, Fig. 1A).[18] Immunoreactivities for NT, AGE, and 4-HNE were frequently recognized in the pseudocalcified foci, neuropil free minerals and a small number of GFSBs. Accumulation of 4-HNE was observed in both disorders in the neurons of the cerebellar dentate nucleus. These findings suggest that lipid peroxidation and protein glycation may be involved in the pallidal and cerebellar degeneration in XPA and CS. On the other hand, only XPA demonstrated nuclear and cytoplasmic accumulation of 8-OHdG and 8-OHG, respectively, in the globus pallidus (Table 1, Fig. 1B).[19] Most XPA cases exhibited reduced cytoplasmic immunoreactivity for Cu/ZnSOD in the cerebral and cerebellar cortical neurons, as well as the basal ganglia (Fig. 1C,D); a few XPA cases also presented reduced immunoreactivity for Mn/SOD in neuronal mitochondria (Table 1). In contrast, five CS cases demonstrated comparatively preserved immunoreactivity for both types of SOD, suggesting that oxidative damage to nucleotides, along with disturbed SOD expression, is involved in

Table 1 Immunohistochemistry summary of xeroderma pigmentosum group A (XPA) and Cockayne syndrome (CS) cases

Case	Age/Sex	Post-mortem time	Brain weight	NT	GFSB	AGE	4-HNE	GFSB	Dentate neurons	8-OHdG	8-OHG	Cu/ZnSOD in the neurons			MnSOD in the neurons		
				Pallidal neuropil		Pallidal neuropil	Pallidal neuropil			Pallidal neurons	Pallidal neurons	Hippo-campus	Cerebellar Coortex	Basal Ganglia	Hippo-campus	Cerebellar cortex	Basal ganglia
Xeroderma pigmentosus group A																	
1	19 y/Male	2 h	580 g	(–)	(–)	1+	(–)	(–)	2+	(–)	1+	1–	1–	1–	1–	1–	1–
2	19 y/Male	2 h	610 g	(–)	(–)	(–)	(–)	(–)	2+	1+	1+	1–	1–	(–)	(–)	(–)	(–)
3	23 y/Female	9 h	580 g	(–)	1+	(–)	(–)	(–)	2+	1+	1+	(–)	(–)	(–)	(–)	(–)	(–)
4	24 y/Female	4 h	500 g	2+	(–)	1+	1+	(–)	(–)	1+	1+	1–	(–)	1–	1–	1–	(–)
5	24 y/Female	5 h	530 g	(–)	(–)	(–)	1+	(–)	2+	1+	1+	1–	1–	1–	(–)	(–)	(–)
Cockayne syndrome																	
6	7 y/Female	4 h	295 g	1+	(–)	2+	2+	2+	1+	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)
7	15 y/Male	4 h	340 g	2+	2+	1+	2+	1+	(–)	(–)	(–)	(–)	1–	(–)	(–)	(–)	(–)
8	16 y/Female	4 h	615 g	2+	1+	1+	1+	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)
9	18 y/Male	3 h	400 g	2+	2+	2+	2+	1+	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)
10	18 y/Male	12 h	414 g	2+	1+	2+	2+	(–)	1+	(–)	(–)	(–)	1–	(–)	(–)	(–)	(–)

Abbreviations: y, years; h, hours; (–), not altered; 1+, mildly accumulated; 2+, intensely accumulated; 1–, reduced; NT, 3-nitrotyrosine; AGE, advanced glycation end products; 4-HNE, 4-hydroxy nonenal; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; 8-OHG, 8-hydroxyguanosine; Cu/ZnSOD, copper/zinc superoxide dismutase; MnSOD, manganese superoxide dismutase.

neurodegeneration of XPA, but not CS, patients. It could be shown by ELISA of urine that there was a remarkable increase above the cut-off value (mean + 2SD in 17 controls) in both urinary 8-OHdG and HEL (Table 2) in an XPA patient (29 years old), who presented with a long survival and complications from diabetes mellitus.[25] Surprisingly, an XPD patient with mild neurological deficits also demonstrated increased urinary 8-OHdG and HEL (Table 2), although the detailed mechanism is not clear.

Spinal muscular atrophy (SMA) and subacute sclerosing panencephalitis (SSPE)

SMA, which is a childhood counterpart of amyotrophic lateral sclerosis that has been linked to chromosome 5, results from the homozygous loss of the survival motor neuron gene 1. SMA has been classified into three clinical groups, depending on the age of onset and achieved motor abilities.[31] The diseases are characterized by progressive loss of motor neurons in the spinal cord and/or brainstem, leading to symmetrical weakness and atrophy in the leg and respiratory muscles. Three cases of type I SMA (Werdnig-Hoffmann disease) and two cases of type II SMA demonstrated an accumulation of 4-HNE in the motor neurons of the hypoglossal nucleus and spinal anterior horn (Table 3, Fig. 2A).[20,21] The atrophic motor neurons in type II SMA cases with a prolonged clinical course also displayed an NT accumulation. Interestingly, oxidative nucleotide damage was observed in the motor cortex, lateral thalamic nucleus, and cerebellar granule cells (Table 3, Fig. 2B). It is likely that oxidative stress might be involved in the degeneration of motor neurons in SMA.

SSPE is a result of persistent mutated measles virus infection of the central nervous system and is characterized by subacute encephalitis, a slow and progressive clinical course, severe brain atrophy, and demyelination of white matter.[32] Isoprinosine and interferon have been reported to provide partial clinical benefits to SSPE patients, although the exact neurodegeneration pathology is still unknown. In six SSPE[22] autopsy cases, measles virus antigen was detected in both neurons and glial cells in cases 1 and 2 having the duration of illness of <3 years, whereas the antigen was detected only in the glial cells in case 3, in which the duration of illness was 9 years. In cases 4, 5, and 6, the duration of illness was ≥10 years, and the measles virus antigen was not identified (Table 4). The nuclei and cytoplasm of the cerebral cortical neurons in cases 1, 2, and 3 were immunoreactive for 8-OHdG and 8-OHG, respectively, whereas the glial cells of the cerebral white matter were immunoreactive for 4-HNE in cases 4, 5, and 6 (Table 4). In SSPE, oxidative nucleoside damage to the cerebral cortical neurons appears to take place during

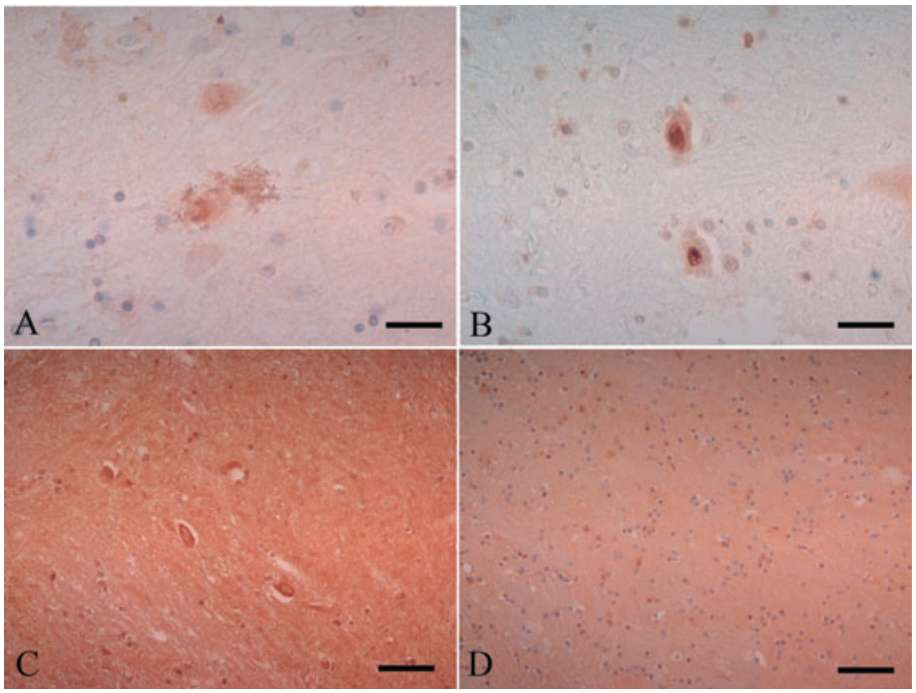

Fig. 1 Representative immunohistochemistry photographs of xeroderma pigmentosum group A (XPA) and Cockayne syndrome (CS) cases. (A) Case 8: 3-nitrotyrosine (NT) immunoreactivity in the neuropil of the internal segment of globus pallidus. (B) Case 4: immunoreactivity of 8-hydroxy-2′-deoxyguanosine (8-OHdG) (D) of neuronal nuclei from the external segment of globus pallidus. Bar = 20 microns. (C) and (D) copper/zinc superoxide dismutase (Cu/ZnSOD) expression in the external segment of globus pallidus in control (C), aged 29 years, and case 4 (D). Bar = 60 microns.

Table 2 ELISA assay summary of xeroderma pigmentosum (XP) patients

Case	Age/Sex	Motor ability	Mental ability	8-OHdG (ng/mg creatinine)	HEL (pmol/mg creatinine)	ACR (nmol/mg creatinine)
XPA	29 y/Male	Bedridden	Respond to pain	23.4↑	254.8↑	190.3
XPA	26 y/Male	Bedridden	Respond to orders	15.3	149.0	76.0
XPA	16 y/Female	Walk with support	Can converse	9.8	139.1	160.4
XPA	7 y/Male	Independent walk	Mild retardation	13.6	117.6	189.2
XPD	9 y/Male	Independent walk	Mild retardation	28.9↑	166.1↑	187.2
Cut off value (the mean+2SD of data in 17 controls)				16.3	144.6	268.9

Abbreviations: XPA, xeroderma pigmentosum group A; XPD, xeroderma pigmentosum group D; y, years; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; HEL, hexanoyl-lysine adduct; ACR, acrolein-lysine adduct.

Table 3 Immunohistochemistry summary of spinal muscular atrophy (SMA) cases

Case	Age/Sex	History of mechanical ventilation	Post-mortem time	Brain weight	NT in the motor neurons	4-HNE in the motor neurons	8-OHdG-immunoreactive nuclei
SMA1							
1	2 m/Male	Absent	2 h	500 g	(–)	(–)	(–)
2	9 m/Female	Absent	2 h	940 g	(–)	(–)	Lateral thalamic nucleus Precentral cortex
3	1 y2 m/Male	Absent	3 h	1000 g	(–)	2+	(–)
4	1 y10 m/Male	Absent	5 h	1090 g	(–)	2+	(–)
5	2 y2 m/Male	Present	3 h	(nd)	(–)	+	Cerebellar granule cells
SMA2							
6	5 y/Female	Absent	2 h	1260 g	+	+	(–)
7	37 y/Female	Absent	10 h	1125 g	+	2+	Lateral thalamic nucleus Cerebellar granule cells

Abbreviations: y, years; m, months; h, hours; (nd), not determined; (–), not altered; 1+, mildly accumulated; 2+, intensely accumulated; NT, 3-nitrotyrosine; 4-HNE, 4-hydroxy nonenal; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

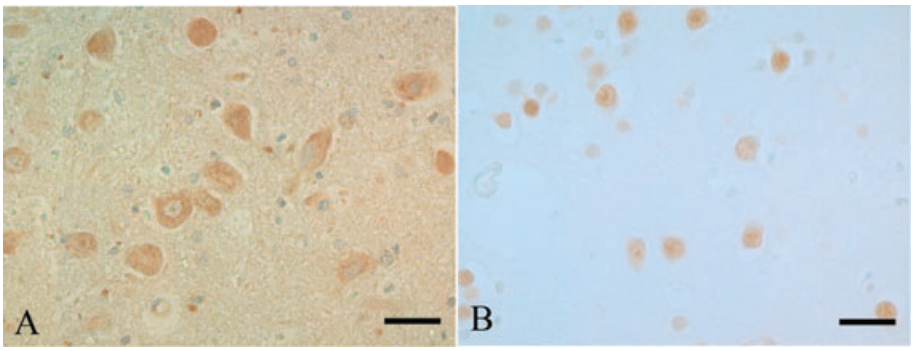

Fig. 2 Representative immunohistochemistry photographs of spinal muscular atrophy (SMA) cases. (A) SMA2 case 7: Accumulation of 4-hydroxy nonenal (4-HNE) in the motor neurons of the hypoglossal nucleus. (B) SMA1 case 2: nuclear immunoreactivity of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in pyramidal neurons of the precentral gyrus. Bar = 30 microns.

Table 4 Immunohistochemistry summary of subacute sclerosing panencephalitis (SSPE) cases

Case	Age/Sex	Disease duration	Post-mortem time	Brain weight	Measles virus antigen	8-OHdG	8-OHG	4-HNE
						Cerebral cortical neurons	Cerebral cortical neurons	Glial cells in the white matters
1	8 y/Male	1 m	2 h	(nd)	Neurons and glial cells	2+	1+	(–)
2	17 y/Male	2 y	2 h	1200 g	Neurons and glial cells	1+	1+	(–)
3	18 y/Male	9 y	2 h	800 g	Glial cells	1+	1+	(–)
4	21 y/Female	10 y	16 h	873 g	(–)	(–)	(–)	1+
5	27 y/Female	18 y	1 h	700 g	(–)	(–)	1+	2+
6	30 y/Female	22 y	2 h	560 g	(–)	(–)	(–)	2+

Abbreviations: y, years; m, months; h, hours; (nd), not determined; (–), not altered; 1+, mildly accumulated; 2+, intensely accumulated; 4-HNE, 4-hydroxy nonenal; 8-OHG, 8-hydroxyguanosine; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

early disease stages, and the subsequent lipid peroxidation in the glial cells might lead to white matter demyelination.

Progressive myoclonic epilepsy (PME)

Progressive myoclonic epilepsy (PME) is characterized by intractable epileptic seizures and neurological deterioration and can be caused by various neurodegenerative disorders and congenital metabolic errors, such as Unverricht-Lundborg disease, LD, NCL, and dentatorubro-pallidoluysian atrophy (DRPLA).[33] The activity of CuZnSOD in blood isolated from PME patients was reported to be significantly reduced, suggesting a possible involvement of oxidative stress in PME pathogenesis.[34] NCL is a group of hereditary, fatal neurodegenerative and lysosomal storage disorders, most of which are clinically manifested by progressive developmental deterioration, visual loss, uncontrolled myoclonic epilepsy, and/or ataxia.[35,36] Originally, NCL was classified into infantile, late-infantile, juvenile, and adult forms; however, several variants have since been reported. We examined five cases of the late infantile form, characterized by a rapid and progressive PME, and two cases of the juvenile form, characterized by the gradual progression of visual disturbances and epilepsy (Table 5).[23,24] Oxidative DNA damage was observed in neurons of the cerebral cortex and/or midbrain in both forms (Table 5). Protein glycation was elevated in cerebellar Purkinje cells and/or midbrain in four of the NCL cases. Lipid peroxidation occurred in the cerebral and cerebellar cortex in both forms as well. These data indicate that oxidative stress to DNA, proteins, and lipids might be involved in neurodegeneration of the cerebral and cerebellar cortex in NCL.

LD is an autosomal recessive disorder with onset in late childhood and is characterized by the presence of intracellular polyglucosan inclusions, called Lafora bodies, in the brain, liver, and skin.[37] Mutations of the EPM2A and NHLRC1 genes have been demonstrated in LD patients. The former gene product laforin is a dual-specific phosphatase, which dephosphorylates complex carbohydrates, while the latter gene product malin is an E3 ubiquitin ligase.[38] Three LD autopsy cases with a family history presented abundant Lafora bodies in the globus pallidus, thalamus, cerebellum, and substantia nigra (Table 6, Fig. 3A). Two of three autopsy cases demonstrated immunoreactivity for 8-OHdG and ACR, predominantly in the cerebral cortex (Fig. 3B); case 1 was also immunoreactive for HEL in the cerebral cortex (Table 6). Two LD patients with *NHLRC1* mutations[26] displayed a mild increase of urinary 8-OHdG (Table 6), but not HEL or ACR, according to the ELISA urine assay. It is likely that oxidative stress to DNA might be involved in neurodegeneration in LD.

Table 5 Immunohistochemistry summary of neuronal ceroid lipofuscinosis (NCL) cases

Case	Age/Sex	Disease form	Myoclonic epilepsy	Visual disability	Post-mortem time	Brain weight	8-OHdG / Cerebral cortex	Midbrain	AGE / Purkinje cells	Midbrain	4-HNE / Cerebral cortex	Purkinje cells
1	8 y/Male	Late infantile	Frequent	Moderate	6 h	(nd)	1+	(–)	2+	(–)	1+	2+
2	10 y/Male	Late infantile	Frequent	Moderate	4 h	445 g	1+	1+	1+	(–)	2+	1+
3	12 y/Male	Late infantile	Frequent	Moderate	4 h	630 g	2+	2+	1+	1+	2+	1+
4	22 y/Male	Juvenile	Present	Moderate	2 h	800 g	1+	(–)	(–)	(–)	(–)	1+
5	55 y/Male	Protracted juvenile	(–)	Severe	3 h	884 g	1+	(–)	2+	2+	1+	(–)

Abbreviations: y, years; h, hours; not determined; (–), not altered; 1+, mildly accumulated; 2+, intensely accumulated; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; AGE, advanced glycation end products; 4-HNE, 4-hydroxy nonenal.

Table 6 Immunohistochemistry and ELISA assay summary of Lafora disease (LD) cases

Case	Age/Sex	Family history	Age of onset	Post-mortem time	Brain weight	Brain regions showing abundant Lafora bodies	8-OHdG	HEL	ACR
Autopsy cases									
1	16 y/Male	Present	12 y	3 h	1400 g	Substantia nigra, Cerebellum Thalamus	Cerebral cortex Lenticulate nucleus	Cerebral cortex	Cerebral cortex
2	24 y/Male	Present	15 y	4 h	1320 g	Substantia nigra, Cerebellum Globus pallidus	(–)	(–)	(–)
3	28 y/Female	Present	12 y	3 h	(nd)	Substantia nigra, Cerebellum Globus pallidus	Cerebral cortex	(–)	Cerebral cortex
Cases with NHLRC1mutations							8-OHdG (ng/mg creatinine)	HEL (pmol/mg creatinine)	ACR (nmol/mg creatinine)
4	24 y/Male	Absent	12 y			(nd)	19.9↑	118.7	142.9
5	32 y/Female	Absent	11 y			(nd)	20.5↑	105.2	173.4
Cut off value (the mean+2SD of data in 17 controls)							16.3	144.6	268.9

Abbreviations: y, years; h, hours; not determined; (–), not altered; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; HEL, hexanoyl-lysine adduct; ACR, acrolein-lysine adduct.

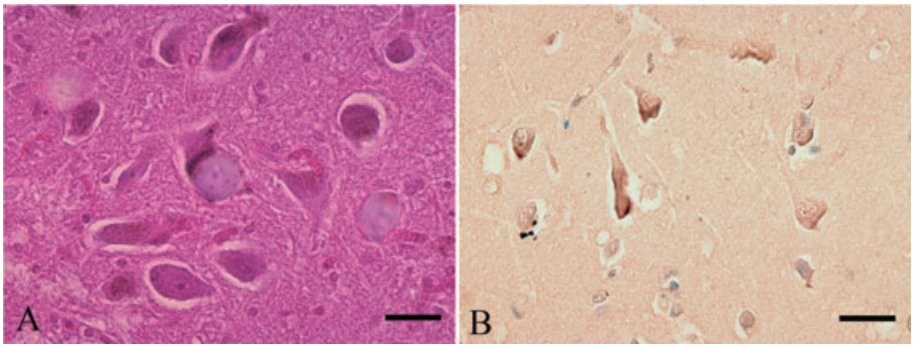

Fig. 3 Lafora body and acrolein-lysine adduct (ACR)-immunoreactivity in Lafora disease (LD) cases. (A) Case 2: Lafora bodies were detected in substantia nigral neurons. (B) Case 1: pyramidal neurons of the parietal cortex were immunoreactive for ACR. Bar = 30 microns.

DISCUSSION

Similar to adult-onset neurodegenerative diseases, oxidative stress could be involved in various child-onset neurological disorders, which suggests a possible utilization of antioxidant agents for treatment of such disorders. Accordingly, we have begun a preliminary trial with vitamins C and E replacement in XPA patients, which received approval from both the family and ethical committees. Edaravone (formerly MCI-186) has been approved as an antioxidant agent for the treatment of acute cerebral infarction, as it inhibits non-enzymatic lipid peroxidation and the lipoxygenase pathway of the arachidonate cascade.[39] In addition, the phase III multi-center clinical trial of edaravone is now in progress in Japan for patients with amyotrophic lateral sclerosis. Because of the present results from these neuropathological analyses, we believe that edaravone could be beneficial for developmental brain disorders and preparations for a phase-III multi-center clinical trial of edaravone, intended for child cerebral infarction, are now underway. The neuropathological analysis and ELISA assay for oxidative stress will be extended to cases with mucopolysaccharidosis and DRPLA. Oxidative stress is one of several factors that can modify neurodegeneration; however, further knowledge of the interactions of oxidative stress with other pathological mechanisms, such as apoptosis, excitotoxicity, and mitochondrial dysfunction, must be further elucidated.

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