CHAPTER 21

OXIDATIVE STRESS IN DEVELOPMENTAL BRAIN DISORDERS

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Abstract: In order to examine the involvement of oxidative stress in developmental brain disorders, we have performed immunohistochemistry in autopsy brains and enzyme-linked immunosorbent assay (ELISA) in the cerebrospinal fluid and urines of patients. Here, we review our data on the hereditary DNA repair disorders, congenital metabolic errors and childhood-onset neurodegenerative disorders. First, in our studies on hereditary DNA repair disorders, increased oxidative DNA damage and lipid peroxidation were carried out in the degeneration of basal ganglia, intracerebral calcification and cerebellar degeneration in patients with xeroderma pigmentosum, Cockayne syndrome and ataxia-telangiectasia-like disorder, respectively. Next, congenital metabolic errors, apoptosis due to lipid peroxidation seemed to cause neuronal damage in neuronal ceroid-lipofuscinosis. Oxidative stress of DNA combined with reduced expression of antioxidant enzymes occurred in the lesion of the cerebral cortex in mucopolysaccharidoses and mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes. In childhood-onset neurodegenerative disorders, increased oxidative DNA damage and lipid peroxidation may lead to motor neuron death in spinal muscular atrophy like in amyotrophic lateral sclerosis. In patients with dentatorubral-pallidoluysian atrophy, a triplet repeat disease, deposition of oxidative products of nucleosides and reduced expression of antioxidant enzymes were found in the lenticular nucleus. In contrast, the involvement of oxidative stress is not definite in patients with Lafora disease. Rett syndrome patients showed changes of oxidative stress markers and antioxidant power in urines, although the changes may be related to systemic complications.

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INTRODUCTION

Oxygen is metabolized to generate energy in the form of ATP through a series of reductive steps at the inner membrane in the mitochondria. During these processes, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are formed. Although ROS and RNS contribute to signal processing, they have also harmful effects on lipids, proteins and nucleic acids, leading to tissue damage in a process called oxidative stress.[1] Oxidative stress originates from an imbalance between the production of ROS and RNS and the antioxidant systems. ROS include superoxide anion ($O_2^{\cdot-}$), hydroxyl radicals (.OH) and hydrogen peroxide (H_2O_2), while nitric oxide (NO) and peroxynitrite ($ONOO^-$) are known as RNS. Antioxidant defenses are composed of preventive antioxidant enzymes such as catalase, superoxide dismutase (SOD), glutathione peroxidase and metal chelating proteins, in addition to radical scavenging vitamins C and E.[2] SOD converts $O_2^{\cdot-}$ into H_2O_2, which is rapidly reduced by catalase and glutathione peroxidase. It may also catalyze excessive nitration of tyrosine by $ONOO^-$. The excess of ROS/RNS production over detoxification results in a shift in balance towards oxidative damage. Oxidative damage of DNA and RNA produces 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 8-hydroxyguanosine (8-OHG), respectively, which are used as markers of oxidative nucleosides damage.[3] Since thymidine glycol (TG) originates from deoxythymidine in DNA but not in RNA and is not removed as easily as 8-OHdG is, TG is a stable oxidative marker specific for DNA.[4] The brain includes a large amount of lipids in cell membranes and the myelin encapsulates the neuronal fibers. Lipid peroxidation can form various aldehydes, including the early and late stage markers hexanoyl lysine adduct (HEL) and 4-hydroxynonenal (4-HNE).[1,5] Advanced glycation end products (AGE) are markers of protein glycoxidation and the generation of AGE has been described in neurological disorders in addition to aging, atherosclerosis and diabetes mellitus.[6]

Oxidative stress markers are also available for the examination of oxidative DNA damage through analysing lipid peroxidation in the urine, serum and cerebrospinal fluid (CSF), using enzyme-linked immunosorbent assay (ELISA) in children.[7-9] Potential antioxidant (PAO) is a marker of antioxidant capacity in various biologic fluids measured by colorimetry, in which Cu^{2+} is reduced by various antioxidants to Cu+. PAO enables the evaluation of not only hydrophilic antioxidants, such as vitamin C and glutathione, but also hydrophobic ones, such as vitamin E.[10] We have confirmed the involvement of oxidative stress in various developmental brain disorders and here we reviewed the data from our immunohistochemical analysis and ELISA.

HEREDITARY DNA REPAIR DISORDERS

DNA damage is implicated in pathogenesis of various neurologic disorders and neurons are targets to sustain DNA damage during oxidative stress.[11,12] Human hereditary DNA repair deficiency syndromes and ataxic disorders seem to provide a hint for linking DNA damage and DNA repair abnormalities with neurodegeneration. Xeroderma pigmentosum (XP) and Cockayne syndrome (CS) are rare, inherited neurocutaneous disorders caused by defects in nucleotide excision repair (NER) system.[13] Complementation studies by using cell hybridization have revealed the existence of eight genes in XP (groups A-G and a variant) and two in CS (A and B). NER includes global genome repair and transcription-coupled repair (TCR), which involves several

XP genes (especially *XP-A* to *XP-G*) and two CS genes (*CSA* and *CSB*). In XP, the disease starts with skin symptoms and progressive neurological manifestations, including cognitive and motor deterioration, neuronal deafness, peripheral neuropathy and brain atrophy occurs more commonly in XP-A, XP-B, XP-D and XP-G.[14] CS children develop severe growth failure with reduced subcutaneous fat, characteristic facial features (sunken eyes, sharp noses and caries teeth), mild skin symptoms and neurological disorders such as demyelinating neuropathy, ataxia, spasticity, deafness and congnitive deterioration.[15] It is likely that decreased DNA repair and persistent DNA damage can result in augmented oxidative nucleotide damage in XP and CS. Oxidative nucleotide damage and antioxidant system have been investigated in isolated skin and blood cells or their cell lines.[14] Nevertheless, protection from ultraviolet (UV) light cannot prevent development of neurodegeneration. We have neuropathologically investigated the deposition of oxidative stress markers in autopsy cases each of XP-A and CS.[16] 4-HNE and, to a lesser extent, AGE were frequently recognized in the pseudocalcified foci, neuropil free minerals and foamy spheroids in the globus pallidus in CS more predominantly than in XP-A. CS cases showed gliosis and calcification in the basal ganglia more remarkably than XP-A cases and the degree of 4-HNE deposition seemed to be in accordance with the calcification. We also found the similar deposition of 4-HNE and AGE in the calcification in the globus pallidus and/or cerebellum in autopsy case each of Fahr disease, pseudohypoparathyroidism and idiopathic intracranial calcification (Fig. 1).[17] Increased oxidative stress has been reported in vascular calcifications in bone and kidney diseases,[18,19] and lipid peroxidation and/or oxidative protein glycation may also affect the calcification subsequent to neurodegeneration in the basal ganglia and cerebellum in the developmental brain disorders including XP-A and CS. Next were examined the deposition of oxidative products in nucleotides and expression of SOD in the XP-A and CS subjects.[20] Cases of XP-A and, to a lesser extent, those of CS demonstrated nuclear deposition of 8-OHdG and TG in neurons and glial cells, in addition to cytoplasmic deposition of 8-OHG, in the globus pallidus and cerebellar cortex (Table 1). Additionally, XP-A cases exhibited reduced cytoplasmic immunoreactivity for Cu/ZnSOD in the neurons of the cerebellar cortex and the basal ganglia, although

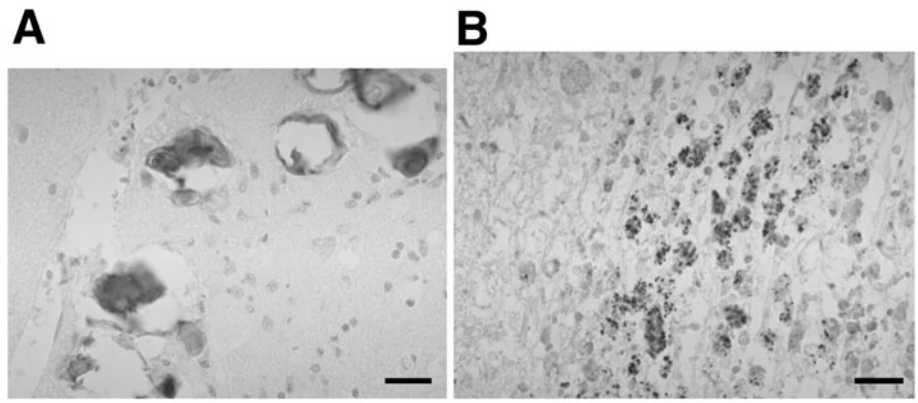

Figure 1. An autopsy case of idiopathic intracranial calcification. A) Perivascular calcification in the cerebellar cortex was immunoreactive for 4-hydroxynonenal Bar = 20 μm. B) Pseudocalcified lesion in the putamen showed granular immunoreactivity for advanced glycation end products. Bar = 20 μm.

Table 1. Summary of Immunohistochemistry for thymidine glycol in autopsy cases of xeroderma pigmentosum group A and Cockayne syndrome.

		Hippocampus		Globus pallidus		Thalamus		Dentate nucleus	
Case	Age/Sex	Neuron	Glia	Neuron	Glia	Neuron	Glia	Neuron	Glia
Xeroderma pigmentosum group A									
1	19 yrs/ Male	2+	2+	(-)	2+	1+	1+	1+	1+
2	19 yrs/ Male	1+	1+	1+	1+	(-)	1+	1+	1+
3	23 yrs/ Female	1+	1+	(-)	2+	1+	(-)	2+	(-)
4	24 yrs/ Female	(-)	(-)	(-)	(-)	(-)	1+	(-)	(-)
5	26 yrs/ Female	(-)	(-)	(-)	1+	(-)	1+	(-)	(-)
Cockayne syndrome									
1	7 yrs/ Female	1+	1+	1+	1+	(-)	(-)	(-)	(-)
2	15 yrs/ Male	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
3	16 yrs/ Female	(-)	(-)	(-)	2+	(-)	2+	(-)	(-)
4	18 yrs/ Male	(-)	(-)	(-)	(-)	(-)	(-)	(-)	1+
5	18 yrs/ Male	(-)	1+	(-)	1+	1+	1+	(-)	(-)

The degree of immunoreactivity for thymidine glycol was graded by the density of positively-stained nuclei of neurons or glial cells ("Glia") according to the following criteria: - = no staining visible, 1+ = a few nuclei were stained, 2+ = many nuclei were stained.

CS cases demonstrated comparatively preserved immunoreactivity for SODs, suggesting that oxidative damage to nucleotides with disturbed SOD expression can be involved in the degeneration of basal ganglia and cerebellum predominantly in XP-A. Next we started the ELISA analysis on 8-OHdG and HEL in urine samples from seven XP-A patients, one XP-D patient, five CS patients and 17 healthy controls aged 3-81 years (Fig. 2). XP-A patients aged over 20 years with long disease duration, suffering from diabetes mellitus and respiratory insufficiency, showed a remarkable increase over the mean of controls in both urinary 8-OHdG and HEL (Fig. 2). In contrast, twin CS patients aged over 20 years showing prolonged disease course demonstrated increased levels of urinary HEL but not urinary 8-OHdG. In the aforementioned autopsy study, markers of oxidative nucleoside damage and those of lipid peroxidation seemed to be deposited in XP-A and CS cases respectively and the similar tendency was speculated in the change of urinary markers from patients with XP-A and CS. In addition, we

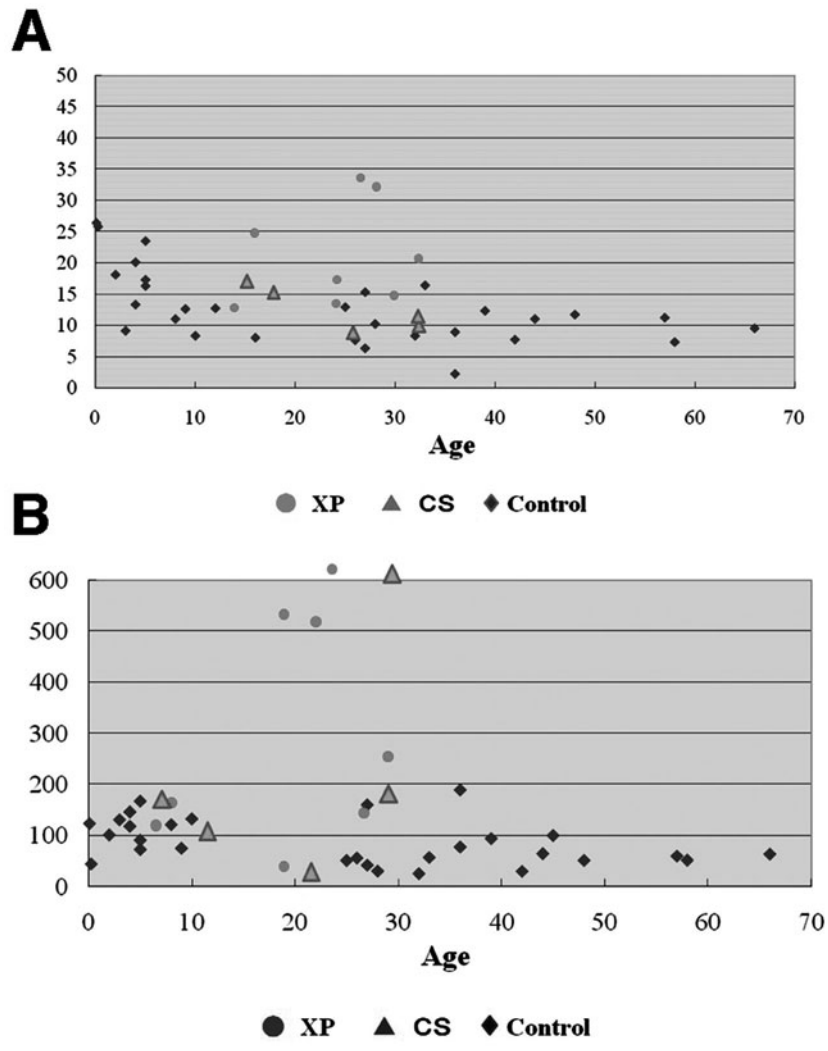

Figure 2. A) Urinary levels of 8-hydroxy-2′-deoxyguanosine (ng/mg Cre.) according to the age (years) in patients with xeroderma pigmentosum (XP, ●) and Cockyane syndrome (CS, ▲), in addition to controls (♦). B) Urinary levels of hexanoyl lysine adduct (pol/mg Cre) according to the age (years) in patients with xeroderma pigmentosum (XP, ●) and Cockyane syndrome (CS, ▲), in addition to controls (♦).

performed a preliminary analysis of the CSF levels of 8-OHdG and HEL in three and one patients of XP-A and XP-D, respectively. One XPA patient showed the increase in level of 8-OHdG in CSF over the cutoff index, whereas the levels of HEL in CSF were not elevated in four patients.

Ataxia-telangiectasia (A-T) is characterized with childhood-onset cerebellar ataxia clinically and progressive atrophy of the cerebellar cortex pathologically. Mutations in the ataxia-telangiectasia mutated (*ATM*) gene give rise to A-T and this gene encodes a protein that is a member of the phosphoinositide 3-kinase family and activation of

ATM by ionizing radiation leads to the phosphorylation of a multitude of substrates involved in recognition of double strand breaks in DNA and in cell cycle checkpoint activation.[21] *Atm*-deficient cells are hypersensitive to oxidative-stress-inducing agents specially the ionizing radiations,[22] and the cerebellum in *Atm*-deficient mice showed progressive accumulation of DNA strand breaks.[23] Antioxidants prevented Purkinje cell death in the aforementioned *Atm*-deficient mice.[24] It is possible that ATM deficiency can enhance oxidative stress and cause oxidative stress-related neuronal death. Nevertheless, oxidative stress has not been examined fully in patients with A-T.[25]

Ataxia-telangiectasia-like disorder (ATLD) is characterized by cerebellar ataxia and ATLD is one of chromosomal breakage syndrome because the patients show spontaneously occurring chromosomal aberrations and increased sensitivity to ionizing radiations.[26] ATLD is caused by mutations in the *MRE11* gene and MRE11 is one of the key components of the signaling network involved in cellular response to DNA damage.[27] We report the neuropathological findings in the first case of genetically confirmed ATLD in a pair of Japanese male siblings.[28] The siblings had the same compound heterozygous mutations of the *MRE11* gene. Brain autopsy demonstrated cerebellar atrophy in the vermis and medial part of the hemispheres, oral to the horizontal fissure. Nuclear immunoreactivity of MRE11 was absent in neurons of cerebellar cortex, cerebral cortex, basal ganglia and midbrain, whereas being widespread in normal control brains. Immunoreactivity of nuclear 8-OHdG was identified in the granule cells and Bergmann glial cells in the cerebellar cortex, both of which were functionally associated with Purkinje cells. Such 8-OHdG expression was absent in the severely affected cerebellar cortex and other brain areas. It is likely that the combination of MRE11 deficiency and oxidative DNA injury may lead to the selective cerebellar damage in patients with ATLD.

CONGENITAL METABOLIC ERRORS

Congenital metabolic errors are composed of heterogeneous diseases, such as lysosomal disorders, mitochondrial encephalomyopathy, peroxisomal disorders and disturbed metabolism of metals, manifesting both neurological and somatic abnormalities. Genes responsible for several diseases have been identified and model animals generated. Nevertheless, the pathogenesis of neurodegeneration still remains to be fully investigated; specific treatments to be developed other than bone marrow transplantation and enzyme replacement, which cannot ameliorate neurological disorders. It will be useful to exploit new therapeutics that can intervene the oxidative stress leading to neuronal damage.

Neuronal ceroid-lipofuscinosis (NCL) is a group of hereditary, lysosomal storage disorders, most of which are clinically manifested by progressive developmental retardation, visual loss, uncontrolled myoclonic epilepsy and/or cerebellar ataxia.[29] NCLs are classically classified into infantile, late-infantile, juvenile and adult forms, but several variants have recently been reported and at least 10 genetically distinct NCLs, designated CLN1 to CLN10, are presently known. We examined three autopsy cases of late-infantile NCL with progressive myoclonic epilepsy (PME), aged 8-12 years,[30] in addition to two autopsy cases of juvenile NCL suffering from the gradual progression of visual disturbances and generalized convulsion.[31] Oxidative DNA damage was observed in neurons of the cerebral cortex and, to a lesser extent, the midbrain, in both types of the disease. Protein glycation was facilitated in the Purkinje cells of the cerebellar cortex in four NCL cases, with the exception of one juvenile case. Lipid peroxidation increased

in the cerebral and cerebellar cortex. Because 4-HNE can activate cell death-related caspases leading to DNA fragmentation, such coexistence of nuclei immunoreactive foe TUNEL and 4-HNE-immunoreactive cytoplasm in the frontal cortical neurons suggested the occurrence of DNA fragmentation triggered by lipid peroxidation in the late infantile form of NCL.

Mucopolysaccharidoses (MPS) are inherited neurodegenerative disorders caused by defects in specific lysosomal enzymes, resulting in the accumulation of undegraded glycosaminoglycans in lysosomes. Sanfilippo syndrome (MPS III) is an autosomal recessive disorder and comprises four subtypes (A, B, C and D), biochemically being linked to different enzymes defects. MPS III Type B (MPS IIIB) is caused by mutations in the gene encoding alpha-*N*-acetylglucosaminidase, a glycosidase required for the degradation of heparin sulfate.[32] The clinical features of MPS IIIB include progressive and profound neurological deterioration, with behavioral disturbances and relatively mild somatic manifestations. No effective therapy has been found yet, although oxidative stress and/or activation of microglia has been suggested to be involved in pathogenesis in model mice.[33,34]

Hunter syndrome (MPS II) is a rare, X-linked disorder caused by a deficiency of the lysosomal enzyme iduronate-2-sulfatase. In the absence of sufficient enzyme activity, glycosaminoglycans accumulate in the lysosomes of many tissues and organs and contribute to the multisystem, progressive pathologies seen in Hunter syndrome.[35] Clinically, MPS II has two subtypes: severe (Hunter A) and mild (Hunter B). Patients with the severe form of MPS II exhibit a chronic and progressive disease involving multiple organs and tissues. Patients with the mild form of MPS II have delayed onset and milder disease progression. Iduronate-2-sulfatase cannot cross the blood–brain barrier and therefore the enzyme replacement therapy is not expected to provide improvement in CNS dysfunction. Neuropathologically, patients with MPS IIIB and MPS II demonstrate neuronal swelling, dilatation of perivascular space, mild gliosis in the white matter and/or hydrocephalus; however, pathogenesis of neurological deterioration remains elusive.[36] The involvement of oxidative damage in the brains of three cases each of MPS IIIB and MPS II and age-matched controls were examined immunochemically.[37] In cases of MPS IIIB, the density of GABAergic interneurons in the cerebral cortex immunoreactive for calbindin-D28K and parvalbumin was markedly reduced when compared with age-matched controls. It was suggested that the disturbance of GABAergic interneurons may be related to mental disturbance. The swollen neurons in the cerebral cortex demonstrated nuclear immunoreactivity for 8-OHdG and apoptotic markers. In contrast, neither lipid peroxidation nor protein glycation were observed in MPS cases. The expressions of Cu/ZnSOD and MnSOD were reduced in two MPS II cases.

Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) are characterized by recurrent stroke-like episodes, epileptic seizure, short stature and deafness. Molecular genetic studies have shown that more than 80% of MELAS patients have the 3243A>G mitochondrial DNA mutation.[38] According to the cohort study in Japan, MELAS is divided into a juvenile form (onset at less than 18 years of age) and an adult form (onset at more than 18 years of age); the former form shows more severe and poor prognosis than those in the latter form.[39] The main neuropathological features of MELAS are infarct-like lesions with necrosis in the cerebral cortex and the adjacent subcortical white matter, subsequent brain atrophy and calcification in the basal ganglia. In the absence of therapeutic intervention, the infarct-like lesions spread into the neighboring region within a few weeks, irrespective of the vascular territory of cerebral

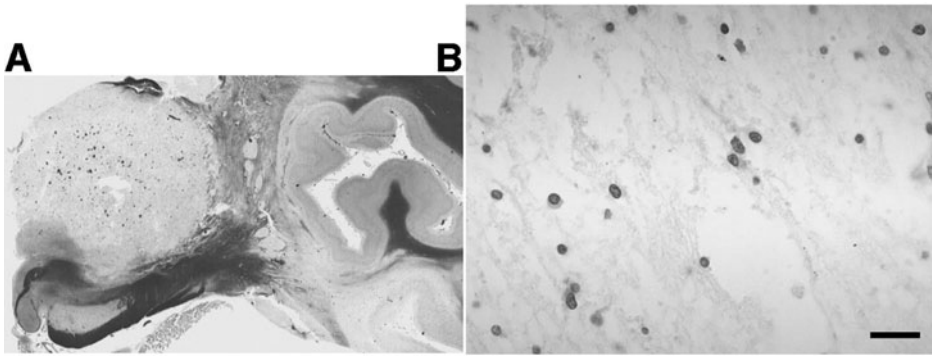

Figure 3. An autopsy case of acute necrotizing encephalopathy caused by influenza infection. A)Severe necrosis in the thalamus, Klüver-Barrera staining. B) Nuclei immunoreactive for 8-hydroxy-2′-deoxyguanosine were found in the glial cells in the necrotic lesion in the thalamus. Bar = 20 μm.

arteries. Even in the cortical and subcortical regions not affected by the infarct-like lesions, there are neuropil micro-vacuolation and increased microvasculature, whereas a few neurons remain in the infarct-like lesions (neuronal sparing). In Japan L-arginine, modulator of vascular endothelial cells, is used for the treatment of MELAS.[39] Abnormal regulation of antioxidants and mitochondrial dysfunction may be involved in oxidative neuronal loss in Huntington's disease and Friedreich ataxia.[40] Therefore, edaravone, a radical scavenger, has been tried to prevent the repetition of stroke-like episodes. The spreading of lesions seemed to occur less frequently in some patients treated with edaravone.[41] Additionally, in the autopsy brains from MELAS cases, having 3243A>G mutation, 8-OHdG was accumulated in the peri-lesional surviving neurons in the cerebral cortex, but the expressions of MnSOD and 8-oxoguanine glycosylase 1 were not up-regulated in those neurons.[41] Increased oxidative stress and insufficient defense could be the reasons of the pathogenesis of the spreading lesions in MELAS. Recently, we found the similar neuropathological characteristics with those in MELAS in the autopsy brains from cases of acute necrotizing encephalopathy caused by influenza infection (iANE).[42] Adjacent to the necrotic lesions in the thalamus and pontine tegmentum, there were neuropil micro-vacuolation, increased microvasculature and neuronal sparing; and nuclei immunoreactive for 8-OHdG was also found in the remaining neurons and glial cells (Fig. 3). In addition, two patients with iANE showed an increased level of 8-OHdG in the CSF. In pathogenesis in iANE, mitochondrial disturbance and/or oxidative stress of DNA is suggested to be involved.

CHILDHOOD-ONSET NEURODEGENERATIVE DISORDERS

Oxidative stress has been confirmed to play an important role in adult-onset neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis.[43] The combination of increased oxidative damage, mitochondrial dysfunction, deposition of oxidized materials, inflammation and defects in protein clearance are most likely reasons of damaging neurons.

Spinal muscular atrophy (SMA) is a childhood-onset motor neuron disease and results from the homozygous loss of *survival motor neuron gene 1* in chromosome 5. SMA is classified into three clinical groups, depending on the age of onset and achieved motor abilities. 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.[44] Three cases of Type I SMA (Werdnig-Hoffmann disease) and two cases of Type II SMA (intermediate type) demonstrated deposition of 4-HNE in the motor neurons of the hypoglossal nucleus and spinal anterior horn.[45,46] Furthermore, nuclei immunoreactive for 8-OHdG were observed in the motor cortex. Also lateral thalamic nucleus and cerebellar granule cells in the absence of neuronal loss and gliosis, indicate that oxidative stress may be the reason in the latent neurodegeneration other than the motor neurons in SMA.

Dentatorubral pallidoluysian atrophy (DRPLA) is a CAG-repeat disease that is classified into juvenile and early adult types showing PME and a late adult type characterized by dementia and cerebellar ataxia.[47] DRPLA patients have an expanded CAG triplet repeat (polyglutamine) on the short arm of chromosome 12 and the degree of polyglutamine expansion is involved in a variety of clinical manifestations. The pattern and distribution of neuropathological changes are region-specific and common in disease types. However intranuclear accumulation of mutant proteins, with expanded polyglutamines is recognized throughout the brain.[48] We examined accumulation of oxidative stress markers and expression of SOD in DRPLA autopsy cases, including four cases of juvenile and late adult types and two cases of early adult type.[49] Neuronal accumulation of 4-HNE was found in the hippocampus, globus pallidus and cerebellar dentate nucleus in the early and late adult types of DRPLA cases. Oxidative products of nucleosides, 8-OHdG and 8-OHG, were accumulated in the lenticular nucleus, predominantly in juvenile and early adult cases showing PME. Mitochondrial immunoreactivity of MnSOD was also reduced in the lenticular nucleus and cerebellum in cases showing PME. Expanded polyglutamine may be the reason for mitochondrial dysfunction and subsequent augmentation of oxidative stress in animal and cell models of DRPLA.[50] It is likely in the juvenile and early adult DRPLA cases the reduced MnSOD expression and increased oxidative DNA damage in the lenticular nucleus may be caused by the expanded polyglutamine, leading to the generation of PME.

Lafora disease (LD) is an autosomal recessive disorder characterized by progressive myoclonic epilepsy and presence of intracellular polyglucosan inclusions, (being called as Lafora bodies) in the brain, liver and cardiac muscles. Mutations of the *EPM2A* and *EPM2B* (*NHLRC1*) genes have been identified in LD patients.[51] Preliminary immunohistochemical analysis was performed in three autopsy cases of LD, which had a family history of LD and the abundant occurrence of Lafora bodies in the globus pallidus, cerebellar dentate nucleus and substantia nigra.[1] Nuclei immunoreactive for 8-OHdG were found in the cerebral cortex in two of three autopsy cases. In addition, two LD patients with *NHLRC1* mutations[52] displayed a mild increase in the level of 8-OHdG in urine, although there was no change in the CSF. Nevertheless, one of the two cases having *NHLRC1* mutations died of cardiac failure at the age of 36 years and immunohistochemistry for oxidative stress markers demonstrated nuclei immunoreactive for 8-OHdG in the neurons of globus pallidus and deposition of 4-HNE in the neuronal cytoplasm in the trochlear and trigeminal nuclei, irrespective of Lafora bodies (Fig. 4). In order to obtain a definite answer on the involvement of oxidative stress in LD, the comprehensive analysis in a large number of patients is necessary.

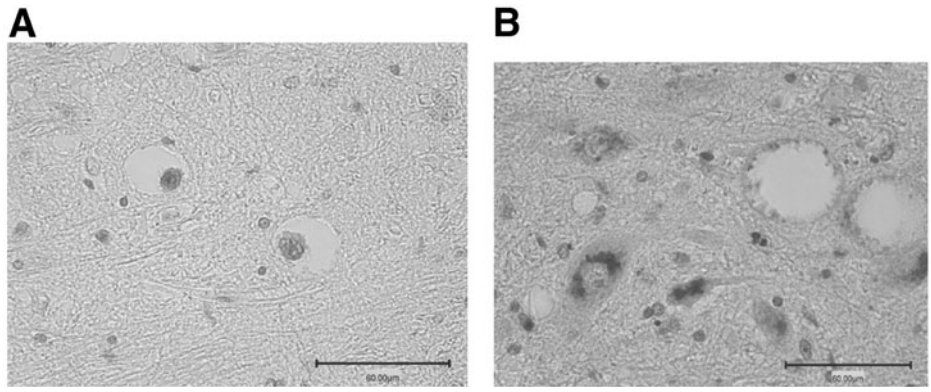

Figure 4. An autopsy case of Lafora disease with mutation of EPM2B gene. A. Nuclei immunoreactive for 8-hydroxy-2′-deoxyguanosine were found in the remaining neurons in the globus pallidus. Bar = 60 μm. B. Neurons with cytoplasm immunoreactive for 4-hydroxynonenal were scattered in the trochlear nucleus. Bar = 60 μm.

Table 2. Urinary levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), hexanoyl lysine adduct (HEL) and potential antioxidant (PAO) in patients with genetically-confirmed Rett syndrome.

		8-OHdG	HEL	PAO
Age	Mutations in *MeCP2* gene	(ng/mg Cre)	(pmol/mg Cre)	(μmol/L aop)
	Cutof f index	16.7	163.9	5187
Younger patients				
7 yrs	R168X	13.8	144.6	7563
10 yrs	R168X	24.6 Δ	153.5	6717
Older patients				
46 yrs	R306C	209.3 ↑	5929 ↑	2440 ↓
46 yrs	Frameshift (at 135)	17.7 Δ	114.7	5761
46 yrs	Frameshift (at 285)	65.3 ↑	453.4 ↑	3443 ↓
47 yrs	T158M	10.3	108.7	5896
49 yrs	R133C	12.6	227 Δ	5164

Cutoff index for each oxidative marker were the mean + 2SD value in controls aged over 6 years. The upward arrows (↑) and triangles (Δ) denote severe and mild increases of 8-OHdG and HEL, respectively, while the downward arrows (↓) mean a decrease of PAO.

Rett syndrome (RS) is a neurodevelopmental disorder mainly caused by de novo mutations in the X-chromosomal *MeCP2* gene encoding the transcriptional regulator methyl-CpG-binding protein 2 and characterized with autistic mental retardation in females.[53] Its pathogenesis remains to be investigated and no effective therapy is available to date.[54] In autopsy brains of autistic cases, oxidative stress is one of possible cause of the Purkinje cell loss in the cerebellar cortex, leading to the cognitive disturbances and several studies have shown decreased levels of antioxidants in blood cells in autistic patients.[55] A few studies on oxidative stress in RS patients, however, demonstrated increased plasma levels of lipid peroxidation markers with reduced activities of the SOD in erythrocytes,[56] and increase of intra-erythrocyte nonprotein-bound iron and protein carbonyl concentrations.[57] We performed preliminary analysis on the levels of 8-OHdG and HEL in the urine of genetically-confirmed RS patients (Table 2). There were no relationships between the phenotype and oxidative stress markers. Older RS patients, having respiratory disturbances, tend to show increased levels of 8-OHdG and/or HEL and lowered antioxidant power. RS patients are known to be associated with several systemic complications, which can alter oxidative stress markers and antioxidant abilities and the analysis in the CSF seems to be prerequisite for further investigation on oxidative stress in neurological disorders in RS.

CONCLUSION

Oxidative stress leading to modification of nucleosides, proteins and lipids may occur in hereditary DNA repair disorders, congenital metabolic errors and childhood-onset neurodegenerative disorders. It is useful for clarifying pathogenesis of neurodegeneration to examine the involvement of oxidative stress, combining immunohistochemistry in the autopsy brains and ELISA in the CSF and urine. Although the involvement of oxidative stress seems to be various, the research indicates that antioxidant therapy may play a major role in alleviating the neurodegeneration in patients with developmental brain disorders.

REFERENCES

1. Hayashi M. Oxidative stress in developmental brain disorders. Neuropathology 2009; 29(1):1-8.
2. Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. Crit Rev Food Sci Nutr 2004; 44(4):275-295.
3. Toyokuni S. Reactive oxygen species-induced molecular damage and its application in pathology. Pathology Int 1999; 49(2):91-102.
4. Iwai S. Synthesis and thermodynamic studies of oligonucleotides containing the two isomers of thymidine glycol. Chemistry 2001; 7(20):4343-4351.
5. Hayashi M, Tanuma N, Miyata R. The involvement of oxidative stress in epilepsy. In: Kozyrev D, Slutsky V, eds. Handbook of Free Radicals: formation, types and effects. New York: Nova Science Publishers, 2010:305-318.
6. Takeuchi M, Yamagishi S. Involvement of toxic AGEs (TAGE) in the pathogenesis of diabetic vascular complications and Alzheimer's disease. J Alzheimers Dis 2009; 16(4):845-858.
7. Shiihara T, Kato M, Ichiyama T et al. Acute encephalopathy with refractory status epilepticus: bilateral mesial temporal and claustral lesions, associated with a peripheral marker of oxidative DNA damage. J Neurol Sci 2006; 250(1-2):159-161.
8. Tanuma N, Miyata R, Hayashi M et al. Oxidative stress as a biomarker of respiratory disturbance in patients with severe motor and intellectual disabilities. Brain Dev 2008; 30(6):402-409.

9. Tanuma N, Miyata R, Kumada S et al. The axonal damage marker tau protein in the cerebrospinal fluid is increased in patients with acute encephalopathy with biphasic seizures and late reduced diffusion. Brain Dev 2010; 32(6):435-439.
10. Izuta H, Matsunaga N, Shimazawa M et al. Proliferative diabetic retinopathy and relations among antioxidant activity, oxidative stress and VEGF in vitreous body. Mol Vis 2010; 16:130-136.
11. Hayashi M, Arai N, Satoh J et al. Neurodegenerative mechanisms in subacute sclerosing panencephalitis. J Child Neurol 2002; 17(10):725-730.
12. Martin LJ. DNA damage and repair: relevance to mechanisms of neurodegeneration. J Neuropathol Exp Neurol 2008; 67(5):377-387.
13. Kraemer KH, Patronas NJ, Schiffmann R et al. Xeroderma pigmentosum, trichothiodystrophy and Cockayne syndrome: a comple genotype-phenotype relationship. Neuroscience 2007; 145(4):1388-1396.
14. Hayashi M. Role of oxidative stress in xeroderma pigmentosum. Adv Exp Med Biol 2008; 637:120-127.
15. Hayashi M, Hayakawa K, Suzuki F et al. A neuropathological study of early onset Cockayne syndrome with chromosomal anomaly 47XXX. Brain Dev 1992; 14(1):63-67.
16. Hayashi M, Itoh M, Araki S et al. Oxidative stress and disturbed glutamate transport in hereditary nucleotide repair disorders. J Neuropathol Exp Neurol 2001; 60(4):350-356.
17. Saito Y, Shibuya M, Hayashi M et al. Cerebellopontine calcification: a new entitiy of idiopathic intracranial calcification? Acta Neuropathol 2005; 110(1):77-83.
18. Mody N, Parhami F, Sarafian TA et al. Oxidative stress modulates osteoblastic differentiation of vascular and bone cells. Free Radic Biol Med 2001; 31(4):509-519.
19. Massy ZA, Maziere C, Kamel S et al. Impact of inflammation and oxidative stress on vascular calcifications in chronic kidney disease. Pediatr Nephrol 2005; 20(3):380-382.
20. Hayashi M, Araki S, Kohyama J et al. Oxidative nucleotide damage and superoxide dismutase expression in the brains of xeroderma pigmentosum group A and Cockayne syndrome. Brain Dev 2005; 27(1):34-38.
21. Ahmad S I (edt). Molecular Mechanisms of Ataxia telangiectasia, Landes Bioscience Publication, 2006.
22. Takao N, Li Y, Yamamoto K. Protective roles for ATM in cellular response to oxidative stress. FEBS Lett 2000; 472(1):133-136.
23. Stern N, Hochman A, Zemach N et al. Accumulation of DNA damage and reduced levels of nicotine adenine dinucleotide in the brains of Atm-deficient mice. J Biol Chem 2002; 277(1):602-608.
24. Chen P, Peng C, Luff J et al. Oxidative stress is responsible for deficient survival and dendritogenesis in Purkinje neurons from ataxiatelangiectasia mutated mice. J Neurosci 2003; 23(36):11453-11460.
25. Russo I, Cosentino C, Del Giudice E et al. In ataxia-teleangiectasia betamethasone response is inversely correlated to cerebellar atrophy and directly to antioxidative capacity. Eur J Neurol 2009; 16(6):755-759.
26. Taylor AM, Groom A, Byrd PJ. Ataxia-telangiectasia-like disorder (ATLD)–its clinical presentation and molecular basis. DNA Repair 2004; 3(8-9):1219-1225.
27. Iijima K, Ohara M, Seki R et al. Dancing on damaged chromatin: functions of ATM and the RAD50/MRE11/NBS1 complex in cellular responses to DNA damage. J Radiat Res 2008; 49(5):451-464.
28. Oba D, Hayashi M, Minamitani M et al. Autopsic study of cerebellar degeneration in siblings with ataxia-telangiectasia-like disorder (ATLD). Acta Neuropathol 2010; 119(4):513-520.
29. Kohlschutter A, Schulz A. Towards understanding the neuronal ceroid lipofuscinoses. Brain Dev 2009; 31(7):499-502.
30. Hachiya Y, Hayashi M, Kumada S et al. Mechanisms of neurodegeneration in neuronal ceroid-lipofuscinosis. Acta Neuropathol 2006; 111(2):168-177.
31. Anzai Y, Hayashi M, Fueki N et al. Protracted juvenile neuronal ceroid lipofuscinosis—an autopsy report and immunohistochemical analysis. Brain Dev 2006; 28(6):462-465.
32. Bunge S, Knigge A, Steglich C et al. Mucopolysaccharidosis type IIIB (Sanfilippo B): identification of 18 novel alpha-N-acetylglucosaminidase gene mutations. J Med Genet 1999; 36(1):28-31.
33. Villani GR, Gargiulo N, Faraonio R et al. Cytokines, neurotrophins and oxidative stress in brain disease from mucopolysaccharidosis IIIB. J Neurosci 2007; 85(3):612-622.
34. Ohmi K, Greenberg DS, Rajavel KS et al. Activated microglia in cortex of mouse models of mucopolysaccharidoses I and IIIB. Proc Natl Acad Sci USA 2003; 100(4):1902-1907.
35. Wraith JE, Scarpa M, Beck M et al. Mucopolysaccharidosis type II (Hunter syndrome): a clinical review and recommendations for treatment in the era of enzyme replacement therapy. Eur J Pediatr 2008; 167(3):267-277.
36. Matalon R, Kaul R, Michals K. Mucopolysaccharidosis and mucolipidosis. In: Duckett S, ed. Pediatric Neuropathology. Baltimore: Williams and Wilkins, 1995:525-544.
37. Hamano K, Hayashi M, Shioda K et al. Mechanisms of neurodegeneration in mucopolysaccharidoses II and IIIB: analysis of human brain tissue. Acta Neuropathol 2008; 115(5):547-559.
38. Iizuka T, Sakai F. Pathogenesis of stroke-like episodes in MELAS: analysis of neurovascular cellular mechanisms. Curr Neurovasc Res 2005; 2(1):29-45.

39. Koga Y, Povalko N, Nishioka J et al. MELAS and L-arginine therapy: pathophysiology of stroke-like episodes. Ann NY Acad Sci 2010; 1201:104-110.
40. Trushima E, McMurray CT. Oxidative stress and mitochondrial dysfunction in neurodegenerative diseases. Neuroscience 2007; 145(4):1233-1248.
41. Katayama Y, Maeda K, Iizuka T et al. Accumulation of oxidative stress around the stroke-like lesions of MELAS patients. Mitochondrion 2009; 9(5):306-313.
42. Mizuguchi M, Hayashi M, Nakano I et al. Concentric structure of thalamic lesions in acute necrotizing encephalopathy. Neuroradiology 2002; 44(6):489-493.
43. Halliwell B. Oxidative stress and neurodegeneration: where are we now? J Neurochem 2006; 97(6):1634-1658.
44. Wirth B, Brichta L, Hahnen E. Spinal muscular atrophy: from gene to therapy. Semin Pediatr Neurol 2006; 13(2):121-131.
45. Hayashi M, Araki S, Arai N et al. Oxidative stress and disturbed glutamate transport in spinal muscular atrophy. Brain Dev 2002; 24(8):770-775.
46. Araki S, Hayashi M, Tamagawa K et al. Neuropathological analysis in spinal muscular atrophy type II. Acta Neuropathol 2003; 106(5):441-448.
47. Hayashi M, Kumada S, Shioda K et al. Neuropathological analysis of the brainstem and cerebral cortex lesions on epileptogenesis in hereditary dentatorubral-pallidoluysian atrophy. Brain Dev 2007; 29(9):473-481.
48. Yamada M. CAG repeat disorder models and human neuropathology: similarities and differences. Acta Neuropathol 2008; 115(1):71-86.
49. Miyata R, Hayashi M, Tanuma N et al. Oxidative stress in neurodegeneration in dentatorubral-pallidoluysian atrophy. J Neurol Sci 2008; 264(1-2):133-139.
50. Puranam KL, Wu G, Strittmatter WJ et al. Polyglutamine expansion inhibits respiration by increasing reactive oxygen species in isolated mitochondria. Biochem Biophys Res Commun 2006; 341(2):607-613.
51. Ganesh S, Puri R, Singh S et al. Recent advances in the molecular basis of Lafora's progressive myoclonus epilepsy. J Hum Genet 2006; 51(1):1-8.
52. Singh S, Suzuki T, Uchiyama A et al. Mutations in the NHLRC1 gene are the common cause for Lafora disease in the Japanese population. J Hum Genet 2005; 50(7):347-352.
53. Gonzales ML, LaSalle JM. The role of MeCP2 in brain development and neurodevelopmental disorders. Curr Psychiatry Rep 2010; 12(2):127-134.
54. Armstrong DD. Neuropathology of Rett syndrome. J Child Neurol 2005; 20(9):747-753.
55. Pardo CA, Eberhart CG. The neurobiology of autism. Brain Pathol 2007; 17(4):434-447.
56. Sierra C, Vilaseca MA, Brandi N et al. Oxidative stress in Rett syndrome. Brain Dev 2001; 23(Suppl 1):S236-S239.
57. De Felice C, Ciccoli L, Leoncini S et al. Systemic oxidative stress in classic Rett syndrome. Free Radic Biol Med 2009; 47(4):440-448.