ELSEVIER

Brain & Development 32 (2010) 435–439

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Original article

The axonal damage marker tau protein in the cerebrospinal fluid is increased in patients with acute encephalopathy with biphasic seizures and late reduced diffusion

Naoyuki Tanuma [a,h,*], Rie Miyata [b,h], Satoko Kumada [c], Masaya Kubota [d], Jun-ichi Takanashi [e], Akihisa Okumura [f], Shin-ichiro Hamano [g], Masaharu Hayashi [h]

[a] *Department of Pediatrics, Tokyo Metropolitan Fuchu Medical Center for the Disabled, Japan*
[b] *Department of Pediatrics, Tokyo Kita Shakai Hoken Hospital, Japan*
[c] *Department of Neuropediatrics, Tokyo Metropolitan Neurological Hospital, Japan*
[d] *Department of Neurology, National Center for Child Health and Development, Japan*
[e] *Department of Pediatrics, Kameda Medical Center, Japan*
[f] *Department of Pediatrics, Juntendo University, School of Medicine, Japan*
[g] *Division of Neurology, Saitama Children's Medical Center, Japan*
[h] *Department of Clinical Neuropathology, Tokyo Metropolitan Institute for Neuroscience, Japan*

Received 16 January 2009; received in revised form 14 June 2009; accepted 7 July 2009

Abstract

Acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) is a recently clinicoradiologically-established encephalopathy syndrome. In the present study, we examined the levels of cerebrospinal fluid (CSF) tau protein, a marker of axonal damage, in 11 patients with AESD. CSF tau levels were normal on day 1 and increased from day 3 of the disease between the initial and the secondary seizures. Magnetic resonance imaging (MRI) reveals reduced diffusion in the subcortical white matter during days 3–7. Two patients showed elevated tau protein prior to the diffusion abnormality of subcortical white matter on MRI. Levels of CSF neuron specific enolase (NSE), a neuronal marker, were elevated in only two out of seven patients with AESD, and CSF tau levels were also increased in these patients. Our results indicated that tau protein is a more sensitive marker than NSE and axonal damage causes the conspicuous MRI findings in AESD patients. A therapeutic strategy for axonal protection should be developed to prevent severe neurological impairment of AESD patients.

Keywords: Acute encephalopathy; Tau protein; Axonal damage

1. Introduction

Tau protein is a microtubule-associated protein found predominantly in neuronal axons. It plays an important role in maintaining the cytoskeleton and axonal transport. It is released into the interstitial fluid compartment during neuroaxonal disintegration and diffuses into the cerebrospinal fluid (CSF). Increased levels of CSF tau are found in various pathological conditions, such as traumatic brain injury (TBI) [1], amyotrophic lateral sclerosis (ALS) [2] and multiple sclerosis (MS) [3–5]. Thus CSF tau protein is considered to be a useful biomarker of axonal damage.

Acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) is recently described by Takan-

* Corresponding author. Address: Department of Pediatrics, Tokyo Metropolitan Fuchu Medical Center for the Disabled, 2-9-2, Musashidai, Fuchu, Tokyo 183-0042, Japan. Tel.: +81 42 323 5115x5232; fax: +81 42 322 6207.
E-mail address: Naoyuki_Tanuma@member.metro.tokyo.jp (N. Tanuma).

0387-7604/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.braindev.2009.07.004

ashi et al. [6,7]. This new encephalopathy syndrome is characterized by a prolonged (>30 min) febrile seizure (FS) on day 1, followed by secondary seizures (most often in a cluster of complex partial seizures) on days 4–6. Magnetic resonance imaging (MRI) shows no abnormality during the first 2 days, but reveals reduced diffusion in the frontoparietal subcortical white matter during days 3–9. This diffusion abnormality disappears between days 9 and 25, resulting in cerebral atrophy, and affected children have subsequently variable levels of neurological sequelae. The cause of the MRI findings in AESD remains unknown, and it is important to diagnose AESD as early as possible because the initial prolonged seizure is not easily distinguishable from convulsive status epilepticus.

In the present study, we examined the levels of CSF tau protein in patients with AESD and found that CSF tau levels were increased from day 3 between the initial and the secondary seizures. Our results suggest that axonal damage is the cause of the MRI findings in AESD.

2. Methods

2.1. Subjects

We studied 11 patients with AESD aged 10 months to 3 years of age (mean age of 1.4 ± 0.7 years) and 31 control children aged 1 month to 16 years (mean age of 3.5 ± 5.4 years). The control group consisted of children who presented with fever and required analysis of CSF to exclude the presence of central nervous system (CNS) infection. They were later found to be free of such infection based on the results of CSF examination and negative clinical findings including febrile convulsions.

The clinical diagnosis of AESD was made according to the biphasic clinical course and characteristic MRI findings [6]. Clinical information on these patients is shown in Table 1. The study protocol was approved by the Human Ethics Review Committee of our institution.

2.2. Sample collection and measurement of CSF tau protein levels

CSF samples were obtained from each subject and immediately stored at −80 °C until analysis. The parents of patients with AESD and normal controls understood the purpose of this study and approved the offer of CSF samples.

Levels of CSF human total tau protein were determined using an enzyme-linked immunosorbent assay (ELISA) kit from BioSource International, Inc., Camarillo, California, USA. Neuron-specific enolase (NSE) levels were measured by a standard radioimmunoassay (RIA). All the analyses were performed in triplicate and in a blinded fashion.

3. Results

3.1. Clinical features of patients with AESD

Infectious agents were identified in 9 out of 11 patients and human herpes virus (HHV)-6 was the most common pathogen (63.6%). The outcome of the 11 patients ranged from almost normal to severe mental retardation.

The representative MRI (Patient 7) was shown in Fig. 1. MRI on day 3 (2 days after the initial prolonged seizure) showed no acute parenchymal lesion in diffusion-weighted image (DWI) (Fig. 1A) or in T2-weighted image (Fig. 1D). Subcortical white matter lesions were observed on day 5 (2 days after the second seizure) and were most conspicuous on DWI (Fig. 1C). The subcortical high signal intensity on DWI disappeared (Fig. 1E) and cerebral atrophy was observed on day 20.

3.2. CSF tau protein levels in normal controls and AESD patients

The kinetics of CSF tau protein levels in normal controls are shown in Fig. 2. In the early infantile period, the level of CSF tau was above 1000 pg/ml (Fig. 2A). This level reduced to below 500 pg/ml at around 1 year of age (Fig. 2B). After 1 year of age, the mean level of CSF tau was 439.6 ± 146.4 pg/ml (mean ± SD) (range; 12–630 pg/ml, $n = 11$).

We next examined the levels of CSF tau protein in patients with AESD (Fig. 3 and Table 1). Although the sampling day of each patient varied from day 1 to day 7, the level of CSF tau was normal on day 1, increased from day 3 and remained above 1000 pg/ml thereafter (Fig. 3). We confirmed elevated tau protein prior to the diffusion abnormality of subcortical white matter on MRI in two patients (Patients 7 and 9).

We also examined the levels of CSF NSE in seven patients with AESD and controls. The average level of CSF NSE in controls is 10.4 ± 3.94 ng/ml (mean ± SD). CSF NSE was elevated in two patients (Patients 8 and 10). Although CSF tau protein level was increased in three (Patients 2, 7 and 9) out of seven patients, CSF NSE was within normal range in these patients.

Regarding the relationship between the level of CSF tau and clinical prognosis, there was no correlation between them (Table 1). Although two patients had high CSF NSE levels, one was without neurological sequelae (Patient 10) and the other had mental retardation (Patient 8).

4. Discussion

In the present study, we demonstrated that the level of CSF tau was elevated in early infantile normal con-

Table 1
Clinical profiles of the 11 patients with AESD.

Patients	Age/sex	Pathogen	Outcome	CSF sampling day	Diffusion abnormality on MRI	Abnormal signal lesion	Tau (pg/ml)	NSE (ng/ml)
1	1y0 m/F	HHV-6	Mild MR (DQ 69.2)	Day 1 Day 3	Day 3	Bil. F. WM	714.5 2376	n.d. n.d.
2	1y4 m/F	Unknown	Psychomotor retardation, epilepsy	Day 1 Day 5	Day 5	Bil. WM (sparing peri-Roland)	412.1 2025.1	4.4 14
3	10mo/F	HHV-6	Without neurological sequelae	Day 1	Day 5	Bil. F. WM	436.7	4.9
4	1y2 m/M	HHV-6	MR (speech delay)	Day 1	Day 5	Bil. F. WM	467	5
5	1y4 m/F	HHV-6	Mild MR (speech delay)	Day 1	Day 6	B. F., T., P., O. WM, GM	379.9	n.d.
6	3y1 m/F	Influenza A	Severe MR (DQ 26), epilepsy	Day 1	Day 7	Bil. F., P., O. WM, GM	416.6	n.d.
7	1y10 m/F	Unknown	Severe MR, hypotonia	Day 3	Day 5	Bil. WM (sparing peri-Roland)	2014.7	9.8
8	1y2 m/M	HHV-6	MR	Day 3	Day 3	Bil. F.	7986.2	25
9	11mo/F	HHV-6	Without neurological sequelae	Day 5	Day 6	RT. F., T. WM	2036	15
10	1y0 m/F	HHV-6	Without neurological sequelae	Day 6	Day 6	Bil. F. WM	1885.6	32
11	1y11 m/M	Influenza B	Without neurological sequelae	Day 7	Day 6	Bil. F. WM	2411.7	n.d.

HHV-6, human herpes virus-6; MR, mental retardation; DQ, Development quotient; Bil., bilateral; rt, right; F., frontal; T., temporal; P., parietal; O., occipital; WM, white matter; GM, gray matter; n.d., not done.
The average level of CSF tau in controls after 1 year of age was 439.6 ± 146.4 pg/ml (mean ± SD) (range; 12–630 pg/ml, $n = 11$).
The average level of CSF NSE in controls is 10.4 ± 3.94 ng/ml (mean ± SD).

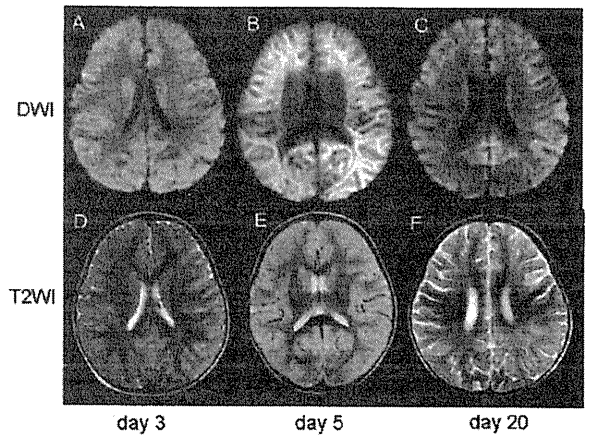


Fig. 1. Representative MRI of a patient with AESD (Patient 7) on days 3 (A and D), 5 (B and E) and 20 (C and E). MRI on day 3 showed no abnormality on diffusion-weighted image (A) or T2-weighted image (D). MRI on day 5 revealed reduced diffusion in the subcortical white matter with sparing of the peri-Rolandic region (B) and T2 prolongation (E). Diffusion-weighted image on day 20 showed mild cerebral atrophy with no signal abnormalities (C). T2-weighted image on day 20 showed T2 prolongation in the subcortical white matter (F).

trols and rapidly reduced after 6–8 months of age. The precise reason for this finding is unknown. However, one possible explanation is that the high level of CSF tau in the early infantile period may be related to myelination, which is most active during this period. Tau protein is also present in oligodendrocytes that make myelin [8,9], and is integral to neuronal process development [10]. Our results suggest that tau protein may be released into the CSF during myelination. Further analysis will be required to confirm this hypothesis.

The most important finding of this study is that the level of CSF tau was normal on day 1 and increased from day 3 between the initial and the secondary (late) seizures in AESD patients. In addition, CSF tau did not increase in patients with febrile seizures without encephalopathy (data not shown). Thus result suggests that the axonal damage in AESD patients begins at least between day 1 and day 3, resulting in a diffusion MRI abnormality thereafter. We also examined NSE, a glycolytic enzyme predominantly present in neurons and endocrine cells. NSE is another index of organic neuronal damage and its CSF levels are increased in several neurological diseases [11,12]. However, the level of

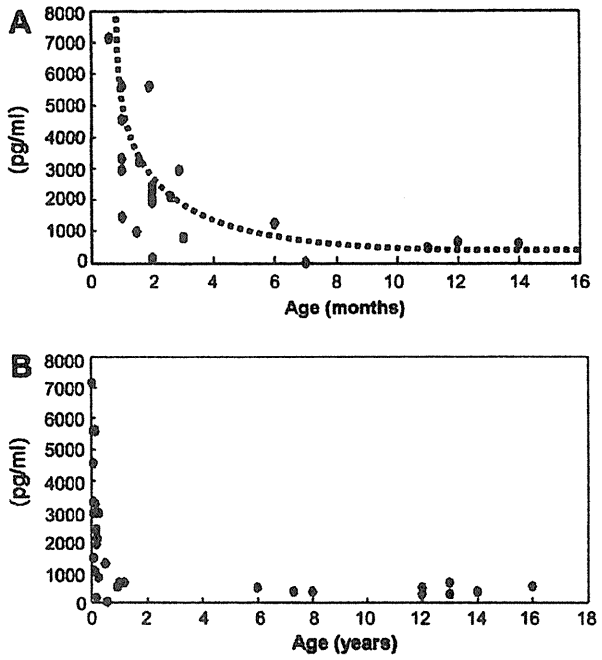


Fig. 2. The kinetics of CSF tau protein levels in normal controls. The level of CSF tau was above 1000 pg/ml in the early infantile period and rapidly reduced to below 500 pg/ml at around 1 year of age (A). Above 1 year of age, the mean level of CSF tau was 363.4 ± 174.7 pg/ml (mean ± SD) (range; 12–618 pg/ml, $n = 11$) (B).

CSF NSE was elevated in only two patients with AESD, although CSF tau levels were increased in patients with normal CSF NSE levels. These results indicate that tau protein is a more sensitive marker than NSE. MRI analysis revealed that reduced diffusion disappears between days 9 and 25, finally resulting in cerebral atrophy. It is likely that axonal damage is the cause of conspicuous diffusion abnormality and late cerebral atrophy in AESD patients.

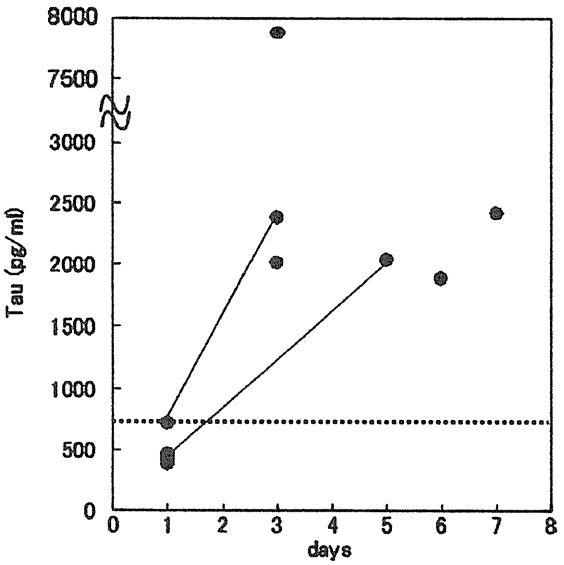


Fig. 3. The levels of CSF tau protein in patients with AESD. The level of CSF tau was normal on day 1, increased from day 3 and remained over 1000 pg/ml thereafter. The dotted line indicates the cut-off value (mean + 2 SD of normal controls). The solid line indicates CSF samples from the same patents (Patients 1 and 2) on a different day.

Although the cause of the MRI findings of AESD remains unknown, excitotoxity is one of the candidates [6,13,14]. MR spectoroscopy revealed that glutamine/glutamate complex (Glx) was elevated during the first week in AESD, although normal Glx concentrations were found in patients with prolonged febrile seizures [6,14]. These findings suggest that excitotoxic neuronal damage is likely to play an important role in the pathogenesis of AESD. The pathomechanisms of excitotoxicity are complex involving glutamate overload, ionic channel dysfunction, calcium overload, mitochondriopathy, proteolytic enzyme production and activation of apoptotic pathways [15]. In addition, glutamate excitotoxicity is considered to be one of the major causes of axonal degeneration in multiple sclerosis [16]. These findings raise the possibility that excitotoxicity may result in not only neuronal damage but also axonal damage in AESD.

Regarding neurological outcome of AESD patients, we first expected that higher tau levels would be associated with more severe brain damage and a worse outcome, because initial ventricular CSF tau levels are correlated with 1-year neurological outcome in patients with traumatic brain injury [1]. Unfortunately, we could not predict the neurological prognosis on day 1 from CSF tau levels. However, three patients with neurological sequelae (Patients 1, 7 and 8) had high CSF tau levels, at least on day 3. By contrast, patients without neurological sequelae (Patients 9, 10 and 11) also had high CSF tau levels, but their levels were elevated after day 5. Thus, it is likely that high CSF levels at an early time point would be associated with a worse outcome. We also examined the levels of CSF tau protein in other type of encephalopathy. Increased CSF tau was also observed in patients with other type of encephalopathy, transverse myelitis and encephalitis (unpublished data), especially in severe cases. In contrast, all the cases in AESD have increased CSF tau after day 3 even if they have no neurological sequelae. It will be useful to further investigate the time course of CSF tau levels in the same patient; alternatively, a larger study would be needed.

Recently, the identification of strategies to protect axons from degeneration in multiple sclerosis patients has emerged as a major research priority [17]. Black et al. reported that the sodium channel blocker phenytoin provides protection for spinal cord axons in long-term monophasic and in chronic-relapsing murine experimental autoimmune encephalomyelitis (EAE) [18] and withdrawal of phenytoin is accompanied by acute exacerbation of EAE [19]. Their results demonstrate the efficacy of phenytoin as a neuroprotectant in

EAE. Intravenous phenytoin is also used in treatment of convulsive status epilepticus (CSE) [20]. In Japan, intravenous phenytoin is less frequently used than midazolam, because of its side effects venous irritation and phlebitis [21]; however, phenytoin (or fosphenytoin) is a second-line antiepileptic drug for CSE in the USA and European countries [20]. These observations together with our results raise the possibility that intravenous phenytoin may prevent axonal damage, if we use it more frequently for the treatment of CSE, especially the initial prolonged febrile seizure in AESD patients. A therapeutic strategy for axonal protection should be developed to prevent severe neurological impairment of AESD patients.

In summary, we demonstrated that CSF tau levels were increased prior to the diffusion abnormality of subcortical white matter on MRI in AESD patients. Our result suggests that axonal damage causes the conspicuous MRI findings.

Acknowledgments

The authors thank A. Koyama, M.D. (Department of Pediatrics, Ome General Hospital), H. Kashii, M.D. (Department of Pediatrics, Tokyo Metropolitan Hachioji Children's Hospital) and A Oba, M.D. (Department of Pediatrics, Matsudo City Hospital) for referring patients. This study was supported in part by the Research Grant from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

[1] Ost M, Nylen K, Csajbok L, Ohrfelt AO, Tullberg M, Wikkelso C, et al. Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury. Neurology 2006;67:1600–4.
[2] Brettschneider J, Petzold A, Sussmuth SD, Ludolph AC, Tumani H. Axonal damage markers in cerebrospinal fluid are increased in ALS. Neurology 2006;66:852–6.
[3] Rostasy K, Withut E, Pohl D, Lange P, Ciesielcyk B, Diem R, et al. Tau, phospho-tau, and S-100B in the cerebrospinal fluid of children with multiple sclerosis. J Child Neurol 2005;20:822–5.
[4] Teunissen CE, Dijkstra C, Polman C. Biological markers in CSF and blood for axonal degeneration in multiple sclerosis. Lancet Neurol 2005;4:32–41.
[5] Terzi M, Birinci A, Cetinkaya E, Onar MK. Cerebrospinal fluid total tau protein levels in patients with multiple sclerosis. Acta Neurol Scand 2007;115:325–30.
[6] Takanashi J, Oba H, Barkovich AJ, Tada H, Tanabe Y, Yamanouchi H, et al. Diffusion MRI abnormalities after prolonged febrile seizures with encephalopathy. Neurology 2006;66:1304–9 [discussion 1291].
[7] Takanashi J, Tsuji M, Amemiya K, Tada H, Barkovich AJ. Mild influenza encephalopathy with biphasic seizures and late reduced diffusion. J Neurol Sci 2007;256:86–9.
[8] LoPresti P, Szuchet S, Papasozomenos SC, Zinkowski RP, Binder LI. Functional implications for the microtubule-associated protein tau: localization in oligodendrocytes. Proc Natl Acad Sci USA 1995;92:10369–73.
[9] Gorath M, Stahnke T, Mronga T, Goldbaum O, Richter-Landsberg C. Developmental changes of tau protein and mRNA in cultured rat brain oligodendrocytes. Glia 2001;36:89–101.
[10] Gordon D, Kidd GJ, Smith R. Antisense suppression of tau in cultured rat oligodendrocytes inhibits process formation. J Neurosci Res 2008;86:2591–601.
[11] Parnetti L, Palumbo B, Cardinali L, Loreti F, Chionne F, Cecchetti R, et al. Cerebrospinal fluid neuron-specific enolase in Alzheimer's disease and vascular dementia. Neurosci Lett 1995;183:43–5.
[12] Palumbo B, Siepi D, Sabalich I, Tranfaglia C, Parnetti L. Cerebrospinal fluid neuron-specific enolase: a further marker of Alzheimer's disease? Funct Neurol 2008;23:93–6.
[13] Mizuguchi M, Yamanouchi H, Ichiyama T, Shiomi M. Acute encephalopathy associated with influenza and other viral infections. Acta Neurol Scand 2007;115:45–56.
[14] Takanashi J, Tada H, Terada H, Barkovich AJ. Excitotoxicity in acute encephalopathy with biphasic seizures and late reduced diffusion. AJNR Am J Neuroradiol 2008;30:132–5.
[15] Gonsette RE. Neurodegeneration in multiple sclerosis: the role of oxidative stress and excitotoxicity. J Neurol Sci 2008.
[16] Werner P, Pitt D, Raine CS. Multiple sclerosis: altered glutamate homeostasis in lesions correlates with oligodendrocyte and axonal damage. Ann Neurol 2001;50:169–80.
[17] Smith KJ. Axonal protection in multiple sclerosis – a particular need during remyelination? Brain 2006;129:3147–9.
[18] Black JA, Liu S, Hains BC, Saab CY, Waxman SG. Long-term protection of central axons with phenytoin in monophasic and chronic-relapsing EAE. Brain 2006;129:3196–208.
[19] Black JA, Waxman SG. Phenytoin protects central axons in experimental autoimmune encephalomyelitis. J Neurol Sci 2008;274:57–63.
[20] Chin RF, Neville BG, Peckham C, Wade A, Bedford H, Scott RC. Treatment of community-onset, childhood convulsive status epilepticus: a prospective, population-based study. Lancet Neurol 2008;7:696–703.
[21] Sugai K. Treatment of convulsive status epilepticus in infants and young children in Japan. Acta Neurol Scand 2007;115:62–70.

doi: 10.1111/j.1346-8138.2009.00608.x

Journal of Dermatology 2009; **36**: 138–143

ORIGINAL ARTICLE

Non-radioisotope method for diagnosing photosensitive genodermatoses and a new marker for xeroderma pigmentosum variant

Satoru HASHIMOTO,[1] Kiyofumi EGAWA,[2] Hironobu IHN,[2] Satoshi TATEISHI[1]

[1]*Institute of Molecular Embryology and Genetics, Kumamoto University, and* [2]*Department of Dermatology, Kumamoto University School of Medicine, Kumamoto, Japan*

ABSTRACT

Xeroderma pigmentosum (XP) is an autosomal recessive disorder characterized by photo-induced deterioration of the skin, which often leads to the early development of skin cancers. To diagnose patients with XP and the related disorder Cockayne syndrome (CS), our laboratory has established a simple autoradiographic method that examines three cellular markers of DNA repair: unscheduled DNA synthesis (UDS), recovery of RNA synthesis (RRS) and recovery of replicative DNA synthesis (RDS). However, it is very laborious to measure the three markers using tritiated thymidine or uridine; therefore, we developed a non-isotope method for diagnosing XP and CS. Fibroblasts from the patient were labeled with bromodeoxyuridine (BrdU) instead of tritiated thymidine to measure UDS and RDS, or were labeled with bromouridine (BrU) instead of tritiated uridine to measure RRS. Incorporated BrdU or BrU could be detected using the immunofluorescence method. Moreover, we discovered a new useful marker for XP variant based on checkpoint activity. The non-radioisotope method and the new marker described here comprise an easy way to diagnose XP and CS.

Key words: cell cycle, Cockayne syndrome (CS), diagnosis, non-radioisotope, xeroderma pigmentosum (XP).

INTRODUCTION

Several genetic disorders result in an increased sensitivity to ultraviolet (UV) due to defects in DNA repair. One of them, xeroderma pigmentosum (XP), is a rare autosomal recessive disorder characterized by photosensitivity, pigment changes, and neoplasias of the skin.[1] In XP patients, skin cancers are approximately 2000 times more frequent than in the general population,[2] and some patients with XP also have neurological complications.

Seven XP complementation groups (XP-A to -G) with defective nucleotide excision repair (NER) are recognized,[3] and a variant form (XP-V) with normal NER but defective translesion DNA synthesis (TLS) caused by mutation in DNA polymerase η, also occurs.[4] NER is an important highly conserved repair system that is responsible for removing several kinds of DNA lesions, particularly those induced by UV irradiation.[5] If not repaired through NER, UV-induced DNA lesions cause mutations during replication, specifically C to T or tandem CC to TT transitions,[6] and lead to variable instability, including epigenetic alterations of oncogenic, tumor-suppressive and cell-cycle control signaling pathways.[7]

Because it is thought that DNA repair interplays with checkpoint activity and apoptosis pathways to maintain genomic stability, we measured the nuclear accumulation of p53 to screen for hereditary photosensitive dermatoses. p53 plays crucial roles in cell-cycle checkpoints, apoptotic cell death and DNA repair, and the nuclear accumulation of p53 after UV irradiation is induced via DNA damage of actively transcribed genes.[8] Moreover, our laboratory has

Correspondence: Satoshi Tateishi, Ph.D., Institute of Molecular Embryology and Genetics, Kumamoto University, 2-2-1 Honjo, Kumamoto 860-0811, Japan. Email: tate@gpo.kumamoto-u.ac.jp

Received 7 January 2008; accepted 8 October 2008.

established a simple method for diagnosing XP and the related disorder, Cockayne syndrome, which involves defects in transcription-coupled NER. The method entails measuring three cellular markers of DNA repair autoradiographically: unscheduled DNA synthesis (UDS), recovery of RNA synthesis (RRS) and recovery of replicative DNA synthesis (RDS).[9] Radioisotope techniques, however, are very laborious. Herein we report an improved fluorescence method and a new useful marker for XP-V based on checkpoint activity after UV irradiation.

METHODS

Cells, culture conditions and UV irradiation conditions

We cultured primary human fibroblasts from three XP-V cells (NPS5, NPS6 and KPS7) assigned by Itoh *et al.*,[9] XP-A cells (KPSX3) and wild-type Turu cells established in this laboratory in Dulbecco's modified Eagle's medium (DMEM; Dainihonnseiyaku, Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS; Thermo Electron, Walthman, MA, USA), penicillin and streptomycin at 37°C under 5% CO_2. We used all cells at less than 10 passages; in these conditions, the growth rates are nearly equal among cells. Cells were irradiated with UV-C using a germicidal lamp (GL-10; Toshiba, Nasu, Japan). The irradiated dose of the UV light was measured with a UVX Radiometer with sensor UVX-25 (UVP, Upland, CA, USA).

Unscheduled DNA synthesis (UDS)

Cells (1×10^5) were seeded on a coverslip (1 cm^2) dipped in culture medium in a 35-mm dish and incubated until subconfluent. They were then washed with phosphate-buffered saline (PBS), irradiated with UV-C at 30 $J \cdot m^{-2}$, labeled for 1 h in medium containing 20 μM bromodeoxyuridine (BrdU) and fixed with 70% ethanol. The samples were denatured with 2 mol/L HCl and washed with PBS. The cells were incubated with diluted anti-BrdU antibody (PharMingen, San Diego, CA, USA) in PBS for 1 h at room temperature, and then washed in PBS and incubated with fluorescein isothiocyanate-conjugated antimouse secondary antibody. After washing the cells on the coverslips, the BrdU incorporated in the cellular DNA was detected under a fluorescence microscope.

Recovery of RNA synthesis (RRS)

Cells (1×10^5) were seeded on three coverslips (1 cm^2) dipped in culture medium in a 35-mm dish and were washed with PBS, irradiated with UV-C at 30 J/m^2 and incubated for various times. Then, bromouridine (BrU) was microinjected into the cytoplasm of the cells, which were incubated for 0.5 h and fixed. The BrU was incorporated into RNA and then detected using the method described for UDS, omitting the denaturing step.

Recovery of replicative DNA synthesis (RDS)

Cells (1×10^5) were seeded on three coverslips (1 cm^2) dipped in culture medium in a 35-mm dish and were treated with or without UV-C radiation at 30 J/m^2, incubated for 2, 8 and 24 h and labeled for 1 h in medium containing 20 μM BrdU. The samples were fixed and denatured. The BrdU incorporated into DNA was detected using the method described for UDS.

Measuring the number of cells in metaphase

Cells (1×10^5) were plated onto three 35-mm dishes. After incubation for 40 h, the cells were irradiated with UV-C at 15 J/m^2 and incubated in the culture medium. The number of round cells as an indicator of metaphases per microscope field (×10) was counted after UV irradiation. We observed 16 fields/plate.

RESULTS

Measuring UDS, RRS, and RDS using fluorescence

Because BrdU incorporated into DNA is easy to detect through immunostaining, we used BrdU to measure the UDS and determined the optimal conditions for measuring the UDS. First, we measured the UDS activity in normal primary fibroblasts (Turu cells) and determined that the optimal conditions consisted of irradiating cells with UV light at 30 J/m^2 and then incubating them with 20 μM BrdU for 1 h. Under these conditions, numerous grains were detected in the nuclei of Turu cells (normal; Fig. 1a), whereas few grains were found in the nuclei of KPSX3 cells (XP-A; Fig. 1b). The defective incorporation of BrdU in the cells was rescued by introducing a plasmid expressing XPA protein, indicating that the grains represent UDS (Fig. 1c). The highly

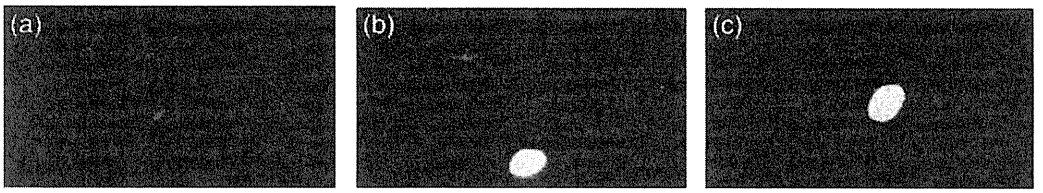


Figure 1. Ultraviolet (UV) induced unscheduled DNA synthesis in normal and xeroderma pigmentosum (XP)-A cells. Cells were irradiated with ultraviolet C at 30 J/m^2, incubated for 1 h with bromodeoxyuridine (BrdU), and processed for immunostaining. (a) Turu cells (normal); (b) KPSX3 cells (XP-A); (c) KPSX3 cells injected with XPA expression plasmid. Bright cells are S-phase cells.

Figure 2. Recovery of RNA synthesis after ultraviolet (UV) irradiation in normal cells. Turu cells were either not irradiated (a) or, irradiated with UV-C at 30 J/m^2 and incubated for 3 h (b) or 24 h (c). The cell cytoplasm was microinjected with bromouridine (BrU), and the cells were incubated for 0.5 h and processed for immunostaining.

fluorescent grains represented S-phase nuclei, which are distinct from the small grains indicating UDS in non-S-phase nuclei, as shown in Figure 1(b,c).

Next, we tried to detect the RRS activity using BrU as an indicator. Numerous grains were observed in the nuclei of Turu cells without UV irradiation (Fig. 2a), and the number of grains decreased dramatically 3 h post-UV irradiation (Fig. 2b). The number of grains recovered by 24 h post-UV irradiation is shown in Figure 2(c).

Because the grains representing the incorporated BrdU in S-phase cells were too numerous to count under the fluorescence microscope (Fig. 1b,c), we determined the RDS by counting the number of BrdU incorporated nuclei per field of UV-irradiated cells, which reflected resumption of DNA synthesis, compared to non-irradiated cells (Fig. 3a). Few BrdU-positive cells were observed at 3 h after UV irradiation in KPS7 cells (XP-V) whereas many Turu cells (normal) were observed. Moreover, this decrease in KPS7 cells continued to at least 24 h after UV irradiation, while the rate of BrdU-positive cells was recovered from approximately 60–90% in Turu cells (Fig. 3b). Interestingly in KPSX3 cells (XP-A), which cells show defective NER but normal TLS, the DNA synthesis rate after UV irradiation was almost the same as Turu cells at 2 h after UV irradiation but declined at 8 and 24 h.

Assessing cell-cycle progression

To confirm the diagnosis of XP-V, the UV sensitivity of cells is generally tested in the presence of caffeine. This process, however, is time-consuming, and so we found a new index for XP-V, a distinct cell-cycle progression following UV irradiation. We determined that testing using this index was rapid and easy. Because it is thought that DNA repair affects cell-cycle progression and that post-replication repair is defective in XP-V cells, while nucleotide excision repair is defective in XP-A through -G, we speculated that a difference exists in the cell-cycle progression among normal, XP-V, and XP-A through -G cells. To test this hypothesis, we examined the number of metaphase cells after UV irradiation (Fig. 4). The number of metaphase cells declined immediately after UV irradiation in all cell types. In XP-A cells (KPSX3) and XP-G cells (data not shown), the number of metaphase cells declined continuously and did not recover, whereas normal cells (Turu) and the three XP-V cells (KPS7, NPS5 and NPS6) recovered to almost the level of non-irradiated cells by approximately 16 h after UV irradiation. However, the XP-V

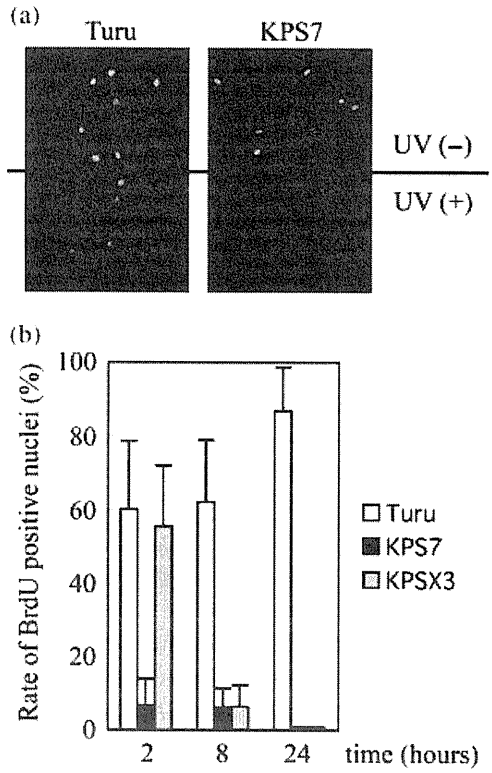


Figure 3. Recovery of replicative DNA synthesis after ultraviolet (UV) irradiation in normal and xeroderma pigmentosum (XP)-V cells. (a) The cells were irradiated with UV-C at 30 J/m^2 or not irradiated, incubated for 2 h, labeled with bromodeoxyuridine (BrdU) for 1 h, and processed for immunostaining. (b) The resumption of DNA synthesis at 2, 8 and 24 h after UV irradiation was calculated as the percentage of BrdU-positive cells versus the percentage without UV irradiation.

cells showed a further decrease in the number of metaphase cells approximately 28–32 h after UV irradiation, while the normal cells maintained cell-cycle progression.

DISCUSSION

We present a simple non-isotope method for diagnosing photosensitive disorders using three markers: UDS, RRS and RDS. Because the genes causing almost all NER- and TLS-deficient disease have been identified, except in UV-sensitive syndrome,[10] it relatively simple to diagnose hereditary photosensitive dermatoses using sequencing analysis. However, unknown disorders may yet exist that are caused by abnormalities in NER or TLS, and UDS,

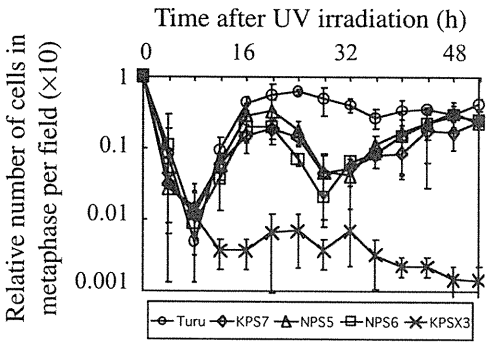


Figure 4. Comparison of the number of cells in metaphase among normal (Turu), xeroderma pigmentosum (XP)-A (KPSX3) and three XP-V (KPS7, NPS5 and NPS6) cells after ultraviolet (UV) irradiation. The cells were seeded on dishes, incubated for 40 h and irradiated with UV-C at 15 J/m^2. The number of cells in metaphase per field was counted after UV irradiation. We observed 16 fields/plate.

RRS and RDS must be assessed in pre-diagnostic screening and as a post-diagnostic complementation assay. To diagnose cells derived from photosensitive patients, our laboratory has established a simple autoradiographic method that examines UDS, RRS and RDS.[9] However, the autoradiographic method is laborious and uses isotopes, which have inherent dangers. These facts impose restrictions on measuring UDS, RRS and RDS in some laboratories. Therefore, we improved the conventional method, and our improved fluorescence method will reduce the labor involved in any laboratories.

Bromodeoxyuridine incorporated into DNA has been used to detect S-phase cells by immunostaining ever since Grantzner[11] established a monoclonal antibody to BrdU. Therefore, we used BrdU to measure UDS and RDS. Although the amount of DNA synthesized in NER was much smaller than that synthesized during replication, the cellular uptake of BrdU was sufficient to detect grains of UDS under the conditions described here. Conversely, in the case of RDS, the cellular uptake of BrdU was too great to count the grains; therefore, we determined RDS by comparing the numbers of BrdU incorporated nuclei of cells following UV irradiation with non-irradiated cells. Similarly, we attempted to detect the RRS activity using BrU. Initially, we used medium containing BrU for labeling, but the cells would not take up BrU. Therefore, we microinjected BrU into the cytoplasm directly and

Table 1. Unscheduled DNA synthesis (UDS), recovery of RNA synthesis (RRS) and replicative DNA synthesis (RDS) measured with conventional radioisotope methods in the xeroderma pigmentosum (XP)-V cells

Cell*	UDS†	RRS‡	RDS§	RDS + caffeine	Reference
KPS7 (XPV)/Mori	126.7 ± 4.9/119.2 ± 5.5	60.5 ± 4.0/53.7 ± 4.2	45.3 ± 4.3/88.6 ± 7.7	18.9 ± 2.8/50.9 ± 4.7	Itoh *et al.*[9]
NPS5 (XPV)/Mori	110.6 ± 7.1/119.2 ± 5.5	58.8 ± 3.7/52.0 ± 4.1	38.9 ± 3.1/61.4 ± 7.2	19.4 ± 3.5/60.8 ± 5.1	
NPS6 (XPV)/Mori	102.3 ± 5.7/122.3 ± 8.8	76.6 ± 4.0/80.7 ± 4.3	43.8 ± 6.1/89.2 ± 7.7	26.7 ± 3.1/62.3 ± 5.3	

*KPS7, NPS5 and NPS6 cells are belonging to xeroderma pigmentosum (XP)-V. Mori is normal human fibroblast. †UDS was measured as follows: the cells were irradiated with ultraviolet (UV) light at a dose of 30 J/m^2, immediately labeled with (^{3}H)thymidine (50 μCi/mL) for 2.5 h. ‡RRS was measured as follows: the cells were irradiated with UV light at a dose of 15 J/m^2, incubated for 23 h and labeled with (^{3}H)uridine (100 μCi/mL) for 1 h. §Recovery of RDS was measured as follows: the cells were irradiated with UV light at a dose of 15 J/m^2, incubated for 6 h and labeled with (^{3}H)thymidine (15 μCi/mL) for 1 h.

were able to detect grains of RRS on the nuclei. However, the RRS technique proposed employs microinjection, which is a highly-skilled technique, not available in some laboratories for routine assay. We assessed these three indexes – UDS, RRS, and RDS with or without caffeine – measured with conventional radioisotope methods using various cells derived from XP variant patients (Table 1) and compared with RDS of KPS7 cells measured by non-radioisotope method (Fig. 3). We further assessed these three indexes – UDS, RRS and RDS – measured with non-radioisotope methods using cells derived from XP patients in addition to this work, two XP-A and each XP-D, XP-G, XP-V and CSB cells, and obtained results similar to those from conventional autoradiography method (data not shown). Moreover, the sensitivity of this assay exhibited no falls negative. Compared with the autoradiography method, our fluorescence method does not seem to be susceptible to quantitation. However, qualitative information of UDS, RRS and RDS is sufficient to be applied to pre-diagnostic screening and post-diagnostic complementation assay. Therefore, we concluded that the non-radioisotope method for measuring UDS, RRS and RDS is reliable for diagnosing XP. Our non-radioisotope method has advantages over the conventional autoradiography method: it is quicker, and most importantly does not use isotopes.

DNA damage induces several responses including DNA repair, checkpoint activity and apoptosis, and checkpoints are associated with biochemical pathways that delay or arrest cell-cycle progression.[12] Because p53 accumulates in the nucleus after various stresses[13] and plays an important role in DNA damage checkpoints, we examined the UV sensitivity and nuclear accumulation of p53 as a way to screen for photosensitive disorders. Because the accumulation of p53 after UV irradiation is induced via DNA damage of actively transcribed genes,[8] we classified photosensitive patients into two groups at screening: the p53 accumulating group, which shows deficient transcription-coupled DNA repair, Cockayne syndrome and XP-A through -G except for -C and -E, while the p53 non-accumulating group includes XP-C and -E. Although XP-V cells accumulate p53, XP-V cells have normal NER. Therefore, we attempted to find a new distinct marker for XP-V.

Xeroderma pigmentosum V patients have mutations in a low-fidelity Y-family DNA polymerase, polymerase η, which is required for bypass replication of UV-induced DNA damage.[4,14] Moreover XP-V cells accumulate in the S-phase after UV irradiation indicating that polymerase η is associated with the S-phase replication checkpoint.[15] The replication checkpoint is thought to prevent the breakdown of the stalled replication fork, and to assist and coordinate the replication fork restart processes by controlling DNA repair, regulating chromatin structure and controlling cell-cycle progression.[16] Therefore, we assessed cell-cycle progression by counting the number of cells in metaphase. XP-V cells exhibited an interesting phenomenon in cell-cycle progression, a fluctuation in the number of metaphase cells (Fig. 4). We believe that the first trough reflects G2/M checkpoint activity. UV-irradiated cells usually arrest cell-cycle progression, which restarts after the DNA is repaired. If the lesion is not repaired, cell death is imminent. Because cells with normal NER are released from cell-cycle arrest at G1/S or G2/M after

completing NER, the number of metaphase cells was restored. In contrast, in XP-V cells, the number of metaphase cells fell again, resulting in a second trough. We attributed this decrease in the number of metaphase cells to an accumulation of S-phase cells by the S-phase checkpoint activation after UV-induced DNA damage in XP-V cells. Consequently, the number of metaphase cells fell again, and XP-V cells showed a unique two-trough phenomenon. Interestingly, these two troughs in XP-V cells can be detected without using caffeine, although it is usually difficult to detect the UV sensitivity of XP-V cells clearly without using caffeine. Therefore, we recommend using this two-trough phenomenon as an easy marker of XP-V. We can identify the second trough by measuring the number of metaphase cells at three points: 16, 28–32 and 48 h after UV irradiation.

ACKNOWLEDGMENTS

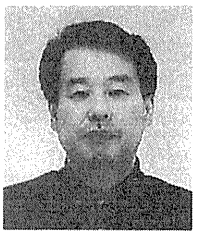
This work was supported by Grants-in-Aids for Scientific Research (nos. 180139 and 18058017) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and a grant from the NOVARTIS Foundation (Japan) for the Promotion of Science.

REFERENCES

1 Kraemer KH, Slor H. Xeroderma pigmentosum. *Clin Dermatol* 1985; **3**: 33–69.
2 Kraemer KH, Lee MM, Scotto J. Xeroderma pigmentosum. Cutaneous, ocular, and neurologic abnormalities in 830 published cases. *Arch Dermatol* 1987; **123**: 241–250.
3 Hanawalt PC. Subpathways of nucleotide excision repair and their regulation. *Oncogene* 2002; **21**: 8949–8956.
4 Masutani C, Kusumoto R, Yamada A *et al*. The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase eta. *Nature* 1999; **399**: 700–704.
5 Prakash S, Prakash L. Nucleotide excision repair in yeast. *Mutat Res* 2000; **451**: 13–24.
6 Afaq F, Adhami VM, Mukhtar H. Photochemoprevention of ultraviolet B signaling and photocarcinogenesis. *Mutat Res* 2005; **571**: 153–173.
7 Hussein MR. Ultraviolet radiation and skin cancer: molecular mechanisms. *J Cutan Pathol* 2005; **32**: 191–205.
8 Yamaizumi M, Sugano T. U.v.-induced nuclear accumulation of p53 is evoked through DNA damage of actively transcribed genes independent of the cell cycle. *Oncogene* 1994; **9**: 2775–2784.
9 Itoh T, Ono T, Yamaizumi M. A simple method for diagnosing xeroderma pigmentosum variant. *J Invest Dermatol* 1996; **107**: 349–353.
10 Itoh T, Ono T, Yamaizumi M. A new UV-sensitive syndrome not belonging to any complementation groups of xeroderma pigmentosum or Cockayne syndrome: siblings showing biochemical characteristics of Cockayne syndrome without typical clinical manifestations. *Mutat Res* 1994; **314**: 233–248.
11 Gratzner HG. Monoclonal antibody to 5-bromo- and 5-iododeoxyuridine: A new reagent for detection of DNA replication. *Science* 1982; **218**: 474–475.
12 Niida H, Nakanishi M. DNA damage checkpoints in mammals. *Mutagenesis* 2006; **21**: 3–9.
13 Sugano T, Nitta M, Ohmori H *et al*. Nuclear accumulation of p53 in normal human fibroblasts is induced by various cellular stresses which evoke the heat shock response, independently of the cell cycle. *Jpn J Cancer Res* 1995; **86**: 415–418.
14 Johnson RE, Kondratick CM, Prakash S *et al*. hRAD30 mutations in the variant form of xeroderma pigmentosum. *Science* 1999; **285**: 263–265.
15 Cleaver JE. Cancer in xeroderma pigmentosum and related disorders of DNA repair. *Nat Rev Cancer* 2005; **5**: 564–573.
16 Branzei D, Foiani M. Interplay of replication checkpoints and repair proteins at stalled replication forks. *DNA Repair (Amst)* 2007; **6**: 994–1003.

色素性乾皮症・Cockayne症候群の神経変性機序と治療の試み

Neurodegeneration in xeroderma pigmentosum and Cockayne syndrome——therapeutic approach



林　雅晴
Masaharu HAYASHI
東京都神経科学総合研究所神経発達・再生研究分野(臨床神経病理)

◎色素性乾皮症(XP)患者では，ヌクレオチド除去修復(NER)の障害により日光過敏症や皮膚癌などの皮膚症状がみられる．XPは遺伝的相補性からNER欠損を伴うA～G群と，NER自体は正常であるバリアント群(XPV)の8つに分類され，日本ではA群(XPA)とXPVが多いといわれる．A・B・D・G群では皮膚症状に加えて神経症状がみられ，なかでも日本のXP患者の約55%を占めるXPAでの神経障害は進行性かつ重篤である．末梢神経・聴神経の障害にはじまり，進行性の知的・運動障害など多彩な神経症状が出現し，生涯にわたる療養が必要となる．日本のXPA患者の80%前後を占めるXPA蛋白遺伝子イントロン3のスプライス異常ホモ接合体の患者では，20歳前後で寝たきりになることが多い．一方，前記異常に別部位の遺伝子変異が組み合わさったヘテロ接合体の患者では寝たきりになるのが30歳前後に遅れる．生存年齢の延長に伴い，発作性の呼吸障害が問題となっている．神経症状は厳重な遮光を行っても年齢とともに進行する．転写鎖修復機構の遺伝的異常により生じるCockayne症候群(CS)においても，皮膚症状に加えて知的障害，小脳失調，難聴，脳内石灰化など多彩な神経症状が出現する．著者らは，XP患者での頭部MRI定量解析や電気生理学的検査，XP・CS患者剖検脳での神経病理学的解析を通じて，神経変性での酸化ストレスの関与，モノアミン神経の選択的障害などを明らかにしてきた．XPの神経症状[1]ならびにXP・CSでの神経病理[2]に関するレビューは報告ずみであり，本稿では，その後の研究により得られた成果と治療的試みについて述べることとする．

Key word：色素性乾皮症，Cockayne症候群，酸化ストレス，レボドパ，アセチルコリン

XP・CS患者での酸化ストレス

酸化ストレスは体内に生じたフリーラジカルを処理できないときに生じ，核酸，脂質，蛋白が障害され，各種の酸化ストレスマーカーが組織に蓄積する．Alzheimer病やParkinson病などで患者脳脊髄液・剖検神経組織での酸化ストレスマーカー蓄積が明らかになっている．

著者らもXPを含む小児の神経変性疾患の剖検脳で酸化ストレスに関する研究に従事し，さらに患者尿・脳脊髄液での酸化ストレスマーカーに関する解析を病理的検討と並行して進めている．大脳基底核の淡蒼球に，XPではDNA，CSでは蛋白・脂質に対する酸化ストレスマーカーがそれぞれ沈着し，さらにCSでは酸化ストレスが石灰化に関連している[3]．そこで大脳基底核に石灰化を示す他の疾患(Fahr病，偽性副甲状腺機能低下症，周産期障害)の剖検脳において，酸化ストレスマーカーに関する免疫組織化学染色を試みた[4]．いずれの病態でもCSと同様に脂質・蛋白に対する酸化ストレスマーカーが石灰化とその近傍に異常蓄積し，酸化ストレスの脳内石灰化への関与が示唆された(図1)．動脈硬化や腎不全においても石灰化に酸化ストレスが関与することが注目されており，今後，CSの腎障害についても酸化ストレス研

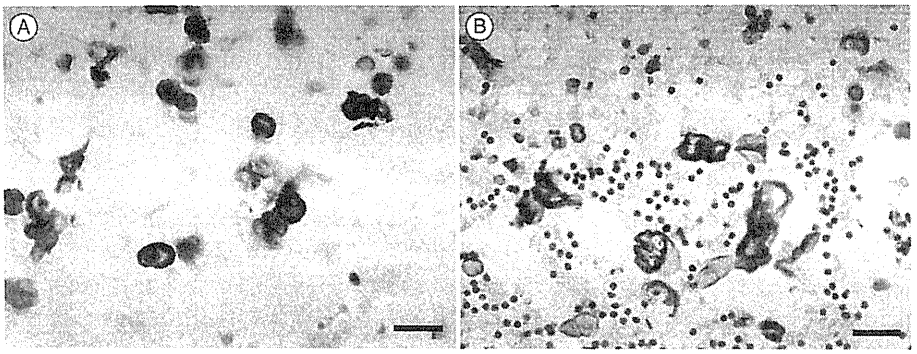


図 1　蛋白に対する酸化ストレスマーカー，Advanced glycation end product免疫組織化学染色
A：18 歳，Cockayne 症候群例の淡蒼球，B：46 歳，偽性副甲状腺機能低下症例の小脳皮質．ともに輪状陽性構造物が石灰化に一致して認められた．バー＝10 μm．

表 1　色素性乾皮症患者の尿中酸化ストレスマーカー

症例	年齢・性	運動機能	聴力障害	呼吸障害	8-Hydroxy-2′-deoxyguanosine (ng/mg creatinine)	Hexanoyl-lysine adduct(pmol/mg creatinine)	Acrolein-lysine adduct(nmol/mg creatinine)
対照(17 例，8〜58 歳)				(平均±SD)	10.5±2.9	69.2±37.7	144.9±62
				(平均＋2 SD)	16.3	144.6	268.9
XPA1	**29 歳・男性	寝たきり	重度	気管切開	23.3↑	188.3↑	221.3
XPA2	26 歳・男性	寝たきり	重度	気管切開	15.3	149↑	76
XPA3	24 歳・女性	寝たきり	重度	気管切開	38.1↑	937.6↑	427.7↑
XPA4	22 歳・女性	介助歩行	中等度	レボドパ	40↑	520.6↑	392.6↑
XPA5	19 歳・男性	車椅子	重度	レボドパ	14.6	43.7	141.4
	18 歳・男性	介助歩行	重度	(中等度)	10.8	(n/a)	203.4
XPA6	19 歳・女性	車椅子	重度	レボドパ	19.5↑	536.6↑	78.2
	**17 歳・女性	介助歩行	中等度	レボドパ	12.7	211.5↑	(n/a)
	**16 歳・女性	介助歩行	中等度	(中等度)	12.8	94.6	215.9
XPA7	7 歳・男性	自立歩行	軽度	(−)	13.6	117.6	189.2
XPD1	9 歳・男性	自立歩行	軽度	(−)	28.9↑	166.1↑	187.2
XP				(平均±SD)	20.9±10.4	296.6±281.5	213.3±116.3

**：本文参照．

究を行う必要性が示唆された．

一方，XP 患者では尿酸化ストレスマーカーの ELISA 法による測定をはじめている[3]．表 1 に XPA 7 例と D 群 XP(XPD)1 例ならびに対照 17 例での解析結果をまとめた．年長例を中心に XPA 7 例中 5 例で脂質に対する早期段階の酸化ストレスマーカー Hexanoyl-lysine adduct(HEL)の上昇がみられ，そのうち 4 例で DNA に対する酸化ストレスマーカー 8-Hydroxy-2′deoxyguanosine (8-OHdG)，2 例で脂質後期段階マーカー Acrolein-lysine adduct がそれぞれ上昇していた．表中“↑”は対照の測定値の平均＋2 SD 以上の高値を意味する．経年的な測定を施行しえた XPA6 では年齢ならびに神経症状の進行に応じた HEL と 8-OHdG の漸増現象がみられた．表中“＊＊”は“一定期間での複数回測定の平均”を意味する．神経症状がそれほど進行していない XPD1 においても HEL と 8-OHdG 上昇が確認された．XP 患者全体での 8-OHdG・HEL 平均値は対照に比べて有意の高値を示した．今後，CS 例でも尿での解析を行うとともに，同意を得られた XP 例において脳脊髄液での酸化ストレスマーカー測定を試みる．将来的には，ビタミン C・E，コエンザイム Q10，エダラボンなどの抗酸化サプリメント・薬物の臨床効果を検証する．

XPA患者での運動療法と少量レボドパ療法

XPA 患者の大脳基底核や脳幹では行動，睡眠，筋緊張，歩行などに関与するカテコールアミン(CA)神経とセロトニン(5HT)神経が選択的に障害されている[5]．小児期において，手と足を組み合わせた左右交互運動が脊髄・脳幹 CA・5HT 神経を刺激し大脳発達を促すことが推定されている[6]．XP 患者においても手と足を組み合わせた左右交互の全身運動(三輪車・自転車，伏し浮き・クロール，起伏がある場所での散歩)を運動機能がある程度保たれている幼少時に行うことは，神経障害進行を遅らせることに役立つ可能性がある．さらに，「お片づけなどの簡単な手伝いを 6 カ月前後継続して行い，記録をとって達成度を確認し，継続できた場合に報酬を与えること」が脳内 CA 神経の発達を促進することが，動物実験や機能 MRI 研究で推定されている[7]．XP 患者においても報酬を伴う家事手伝いを積極的に試みるべきである．

前記のように年長 XPA 患者では睡眠時無呼吸，声帯麻痺，喉頭ジストニアなどが関与して，食事中・夜間に発作性呼吸障害が出現する[1]．数年継続した後，自然に消失することもあるが，気管切開を必要とすることが多い．声帯麻痺は脳幹障害や迷走神経麻痺によるものと推定され，一方，喉頭ジストニアは声帯外転筋・内転筋が同時収縮し吸気時に声帯が閉鎖する不随意運動であり，成人の大脳基底核疾患でよく認められる．ボツリヌス毒素の声帯投与や甲状軟骨部分切開術が行われるが，いずれも侵襲性が高く XP 患者には適さない．

瀬川小児神経学クリニックでは，重症チックの患者に対して少量レボドパ療法(通常量 20～30 mg/kg/day に対し 0.5～1.0 mg/kg/day)を行い，半数弱の症例で効果を認めている[8]．重症チックでは大脳基底核ドパミン神経受容体の過感受性が関与し，少量レボドパ投与によりその過感受性が緩和されチックが改善すると推定される．投与初期に 5～10％の患者でチックの一過性増悪がみられる以外は重篤な副作用は通常生じない．

XP の大脳基底核では黒質からのドパミン神経入力が選択的に障害され，ドパミン神経受容体の過感受性が喉頭ジストニアや四肢の振戦・ミオクローヌス様不随意運動に関与している可能性が想定された．そこで著者らは，XPA 患者 6 名で少量レボドパ療法を試み(0.3～1.0 mg/kg/day)，2 例の患者で呼吸障害の軽減と喉頭ファイバー検査上の声帯運動の改善を確認し，少量レボドパ療法が喉頭ジストニアに有効であることを明らかにした．さらに，この 2 例では上肢のミオクローヌスならびに振戦も軽減し，少量レボドパ療法が XPA での不随意運動にも効果がある可能性が示唆された．ただし，レボドパ開始初期に筋緊張の異常低下がみられることがあり，3 例では投与初期でのレボドパ中止を余儀なくされた．少量レボドパ療法に伴う筋緊張低下は他疾患ではみられない現象であり，大脳基底核ドパミン受容体活性の調整により筋緊張亢進が抑制された結果，XPA に合併する末梢神経障害や小脳失調に伴う筋緊張低下がめだつようになったと推定される．

XPAでのアセチルコリン神経障害

中枢神経系のアセチルコリン(Ach)神経は Meynert 核(MyN)を含む前脳基底部細胞群と脚橋被蓋核(PPN)を中心とする脚橋・被蓋細胞群に 2 大別される．PPN は大脳基底核・視床を介して大脳皮質と連絡し，レム睡眠，筋緊張形成，歩行などに関与する．MyN からは大脳皮質に Ach 作動性線維が投射され，知能・学習に関係する．青斑核の CA 神経と縫線核の 5HT 神経は覚醒時に活動が高まり，レム睡眠時に活動停止する．一方，PPN の Ach 神経はレム睡眠時に活動が高まり覚醒時に活動停止する[9]．Alzheimer 病では大脳皮質に入力する Ach 神経が障害されるため，Ach 作動性の塩酸ドネペジル(商品名：アリセプト®)が汎用され，認知機能，レム睡眠の減少，睡眠時無呼吸などに有効であることが知られている[10]．

著者らは正常対照剖検脳での PPN を解析し，乳児期から Ach 神経(5～25％)と CA 神経(5～30％)が同定されることを明らかにした[11]．Ach 神経の比率は 40 歳未満では 15～25％であったが，60 歳以上で 10％以下に低下し，加齢性変化が推定された．一方，CA 神経の比率は 40 歳未満で 16％以下，50 歳以上で 20％以上と逆に増加傾向を示した．周産期障害である核黄疸後遺症と仮死後遺症で PPN を検討したところ，半数例で PPN の Ach 神

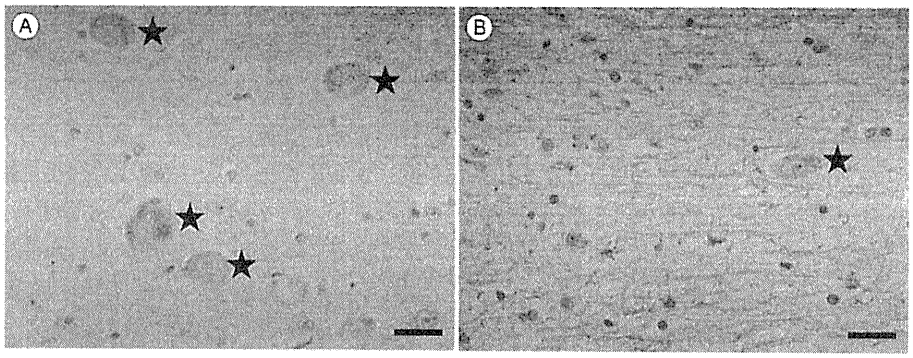


図 2　Meynert核のacetylcholine esterase陽性細胞
A：29 歳，対照，B：21 歳，A 群色素性乾皮症(XPA)．
XPA 脳では胞体に陽性顆粒を有するアセチルコリン神経細胞(★)が減少していた．
バー＝10 μm．

経が減少し，さらに核黄疸後遺症例では CA 神経の代償性増加がみられ，レム睡眠指標の異常との関連が示唆された[12]．

続いて，XPA 剖検脳において Ach 神経病変の解析を行った．XPA 6 剖検例(19〜26 歳)と，神経系に異常を認めない年齢相当対照 6 例(4 カ月〜38 歳)で，acetylcholine esterase(AchE)，tyrosine hydroxylase(CA 神経指標)，calbindin D28K に対する免疫組織化学染色を行った[13]．全例の MyN と PPN で calbindin D28K 陽性 GABA 系神経細胞が同定された．それに対して AchE 陽性神経細胞の総数は，MyN：対照 91.7±25.8 に対して XPA 0.4±0.8，PPN：対照 68.2±18 に対して XPA 0.2±0.4 であり，両部位ともに高度の減少を示した(図 2)．Tyrosine hydroxylase 陽性神経細胞も大脳基底核・脳幹に関する既報告[5]のように減少し，核黄疸後遺症例のような Ach 神経減少に対する代償性増加はみられなかった．成人発症の神経変性疾患で注目されているリン酸化タウ蛋白とリン酸化 α-シヌクレイン蛋白に関する免疫組織化学染色も行ったが，大脳辺縁系を含めいずれの部位にも神経細胞での異常蓄積を確認できなかった．XPA 患者は知的障害のみならず，睡眠時無呼吸やレム睡眠指標の異常を呈することが知られており[14]，今回明らかになった MyN と PPN の Ach 神経病変との関連が示唆された．Alzheimer 病で使用されている Ach 作動性の塩酸ドネペジルが XPA 患者の知的障害やレム睡眠異常に対しても有用である可能性が示唆された．

文献

1) 宮田理英・他：色素性乾皮症の神経症状．医学のあゆみ，**214**：201-204，2005.
2) 林　雅晴：色素性乾皮症と Cockayne 症候群の神経病理．医学のあゆみ，**214**：205-208，2005.
3) Hayashi, M.：Role of oxidative stress in xeroderma pigmentosum. *In*：Molecular Mechanisms of Xeroderma Pigmentosum(ed. by Ahmad, S. I. and Hanaoka, F.). Landes Bioscience, New York, 2008, pp.120-127.
4) 林　雅晴・他：脳基底核・小脳石灰化における酸化ストレスの関与．*Neuropathology*, **28**(Suppl.)：125, 2008.
5) Hayashi, M. et al.：Brainstem and basal ganglia lesions in xeroderma pigmentosum group A. *J. Neuropathol. Exp. Neurol.*, **63**：1048-1057, 2004.
6) 瀬川昌也：Locomotion の発達とその異常(Ⅰ)．臨床脳波，**41**：385-391，1999.
7) Walker, M. A.：Treatment of autism spectrum disorders：neurotransmitter signaling pathways involved in motivation and reward as therapeutic targets. *Expert. Opin. Ther. Targets*, **12**：949-967, 2008.
8) 野村芳子：Gilles de la Tourette 症候群の症候．神経内科，**62**：133-139，2007.
9) 小山純正：睡眠を調節する機構(2)．臨床脳波，**46**：111-117，2004.
10) Moraes, W. et al.：Donepezil improves obstructive sleep apnea in Alzheimer disease. *Chest*, **133**：677-683, 2008.
11) Anzai, Y. et al.：The pedunculopontine nucleus in developmental disorders of the basal ganglia. *Neuropathology*, **28**：258-263, 2008.
12) Hayashi, M. et al.：REM sleep abnormalities in severe athetoid cerebral palsy. *Brain Dev.*, **12**：494-497, 1990.
13) 林　雅晴：色素性乾皮症の神経障害に関する臨床神経病理学的研究．厚生労働科学研究費補助金難治性疾患克服研究事業「神経皮膚症候群に関する調査研究」，平成 19 年度総括・分担研究報告書，2008, pp.48-49.
14) Kohyama, J. et al.：Motor disturbance during REM sleep in group A xeroderma pigmentosum. *Acta Neurol. Scand.*, **92**：91-95, 1995.