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CASE REPORT

Minocycline for the treatment of sarcoidosis: is the mechanism of action immunomodulating or antimicrobial effect?

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Abstract A 47-year-old female was diagnosed to have pulmonary, ocular, and nodular-type muscular sarcoidosis. Seven years later, nodules developed in all limb muscles. She received minocycline 200 mg daily, which resulted in an obvious reduction of the muscular sarcoidosis with a significant decrease in the serum angiotensin-converting enzyme level. Nine months later, the minocycline was discontinued, thus resulting in a rapid recurrence of the disease. The immediate readministration of minocycline again resulted in a prompt improvement. We detected *Propionibacterium acnes* within the granulomas in the affected muscle by an immunohistochemistry. More interestingly, we found a decrease in the circulating levels of interleukin-12 p40 and interferon-inducible protein-10 during the minocycline therapy. The minocycline therapy may be effective for sarcoidosis and the fact that the disease rapidly relapsed after discontinuation of the minocycline administration suggests that the mechanism of action in this case may be immunomodulating but not antimicrobial effect.

Keywords Chemokine · Minocycline · *Propionibacterium acnes* · Sarcoidosis · Treatment

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Introduction

Minocycline has been used as an antimicrobial agent, and recently its anti-inflammatory activities were applied to several kinds of inflammatory diseases. Some authors indicate the effectiveness of minocycline for the treatment of cutaneous sarcoidosis [1] and ocular sarcoidosis [2]; however, the mechanism of action and the long-term efficacy of minocycline therapy have not been fully understood.

Here, we report a patient with pulmonary, ocular, and muscular sarcoidosis developing in all limb muscles. The patient received 200 mg daily of minocycline, which achieved a partial improvement of muscular sarcoidosis, but she experienced a rapid relapse of the disease with the discontinuance of the drug.

Case report

A 47-year-old woman complained of hazy vision and she was diagnosed as having uveitis in 1997. One year later, she noted a palpable nodule in her right leg and therefore she was admitted to our hospital for further evaluation. The chest X-ray film showed bilateral hilar lymphadenopathy (BHL). The diagnosis of sarcoidosis was made on the basis

of uveitis, BHL, lymphocytosis with an increase of CD4/8 ratio in bronchoalveolar lavage fluid and the detection of noncaseating epithelioid cell granulomas by muscle biopsy. At that time, the levels of angiotensin-converting enzyme (ACE) and lysozyme were low with values of 13.1 IU/l (normal, <21.6 IU/L) and 9.7 μg/ml (normal, <10.1 μg/ml). Magnetic resonance image (MRI) showed a nodular bright signal on T2-weighted images, which was enhanced by gadrinium injection. ^{67}Ga citrate scintigraphy showed intensely increased nodular activity in the right leg.

Then, palpable nodules gradually increased and developed in all limb muscles by 7 years. Serum levels of ACE, lysozyme, and soluble interleukin-2 receptor (sIL-2R) rose to 74.6 IU/l, 46.0 μg/ml, and 3,924 U/ml, respectively. Nevertheless, she complained of expansion in her legs without muscle weakness. We observed diffuse accumulation in both upper and lower extremities in ^{67}Ga scintigraphy and numerous intramuscular nodules of high signal intensity on MRI of both legs. The levels of creatine kinase, aldolase, and myoglobin were normal at 76 IU/l, 5.4 IU/l, and 10 ng/ml, respectively. The peripheral white blood cell count was 4,400/mm^3, with 70.1% neutrophils, 13.7% lymphocytes, 10.3% monocytes, and 1.6% eosinophils. Serum level of aspartate transminase was 19.5 IU/l, alanine transminase 11.2 IU/l, lactate dehydrogenase 227 IU/l, blood urea nitrogen 9.5 mg/dl, calcium 9.34 mg/dl, and C-reactive protein 0.02 mg/dl. Chest X-ray showed mild BHL but not pulmonary infiltrates. Cardiac examination by electrocardiogram and echocardiogram was normal.

As she refused an administration of corticosteroid, therapy was initiated with minocycline 200 mg daily. Four months later, her muscle symptoms were reduced and the levels of ACE, lysozyme, and sIL-2R significantly decreased. Nine months later, because the ACE level appeared to reach a minimum value, minocycline was discontinued. In a month, she again felt muscle expansion in her limbs with an increase of serum ACE, lysozyme, and sIL-2R. Minocycline was therefore readministered, resulting in prompt improvement of symptoms and decrease in ACE, lysozyme, and sIL-2R levels. After the second course of minocycline therapy, bright signals on MRI images were diminished (Fig. 1a,b) and Ga scintigraphy revealed decreased activity. During a long-term therapy with minocycline, there were no serious symptoms and laboratory abnormalities although the patient noticed hyperpigmentation in her nails.

To examine the presence of *Propionibacterium acnes* in the granulomatous lesions, the muscle biopsy specimens were served for immunohistochemistry with a specific monoclonal antibody against *P. acnes*, which detected numerous small particles within granuloma macrophages and giant cells (Fig. 1c). We also measured circulating cytokine and chemokine levels during the course of minocycline therapy. Levels of interleukin-12 (IL-12) p40 and interferon-inducible protein-10 (IP-10), which are associated with T helper type 1 (Th1) response, were elevated before minocycline administration and decreased during the therapy. In contrast, levels of thymus- and activation-regulated chemokine, Th2-associated chemokine, were kept elevated during the course of minocycline treatment.

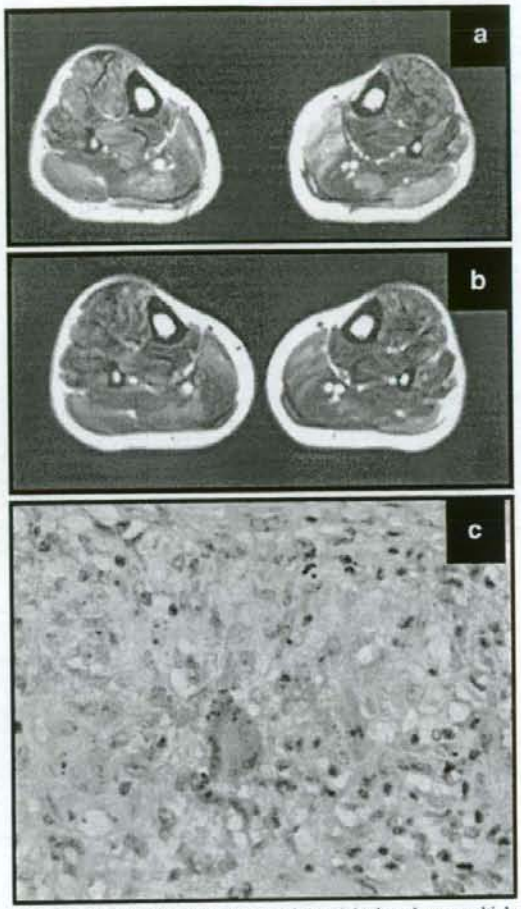

Fig. 1 Magnetic resonance image of the right leg shows multiple bright signals on T2-weighted images (**a**), and after minocycline therapy, the bright signals are decreased (**b**). **c** Immunohistochemical staining with a specific monoclonal antibody against *P. acnes* detects numerous small particles within the granuloma macrophages and giant cells

Discussion

We found during the minocycline treatment a regression of nodular lesion of muscular sarcoidosis with a decrease in levels of ACE, lysozyme, and sIL-2R. Because serum

levels of ACE reflect granuloma load, significant decrease of the serum marker indicated diminished granulomatous inflammation caused by minocycline. Thus, the effectiveness of minocycline for muscular sarcoidosis in this case was confirmed when a prompt response to the minocycline therapy was repeatedly observed.

The mechanism of action in the minocycline therapy remains controversial. In the previous study, ribosomal RNA fragments of *P. acnes* were detected in most lymph-node specimens from sarcoidosis patients [3]. The presence of *P. acnes* within the sarcoid granuloma was detected by an immunohistochemistry in this case; therefore, someone may consider that clinical improvement might be due to the antimicrobial effect of minocycline. However, the fact that the disease rapidly relapsed after discontinuation of minocycline in this case suggests that the mechanism of action is immunomodulation and not antimicrobial effect. It was unfortunate that we could not examine for the presence or absence of *P. acnes* after the therapy because the patient refused to allow a repeat biopsy.

Minocycline suppresses in vitro granuloma formation by monocytes exposed to dextrin beads [4] and also has potency to inhibit T-lymphocyte activation and proliferation [5]. Recent report demonstrated that minocycline therapy suppressed the expression of T-lymphocyte-associated chemokines and chemokine receptor CXCR3 which exists on Th1 cells [6]. During the minocycline therapy for our patient, we found a decrease of serum levels of IP-10, a ligand of CXCR3, as well as IL-12p40. Both IL-12 and IP-10 are generated by granuloma macrophages at the inflamed sites and moved into blood stream, thus reflecting disease activity of sarcoidosis [7, 8]. Given the relationship between minocycline administration and the changes in circulating levels of IP-10 and IL-12p40 in this patient, minocycline therapy might suppress IP-10 and IL-12p40 production by granuloma macrophages at the sites of inflammation.

The present case may also suggest the limitation of minocycline single therapy because minocycline failed to achieve a complete remission and more importantly discontinuing minocycline induced a recurrence. Bachelez et al. [1] previously treated 12 patients with cutaneous sarcoidosis with minocycline over a median period of 12 months. With a median follow-up of 26 months, the authors noted complete and partial responses to treatment in eight and two patients, respectively. They also mentioned that recurrence occurred in three of seven patients after discontinuing the drug. Further studies including randomized controlled trials are needed to assess the long-term efficacy of minocycline for sarcoidosis.

Disclosures None.

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Prognostic Factors in Influenza-associated Encephalopathy

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Background: Recently, reports of influenza-associated encephalopathy have increased worldwide. Given the high mortality and morbidity rates attributable to this severe neurologic complication of influenza, we conducted a nationwide study in Japan to identify the prognostic factors.

Methods: We retrospectively evaluated 442 cases of influenza-associated encephalopathy that were reported to the Collaborative Study Group on Influenza-Associated Encephalopathy, which was organized by the Japanese Ministry of Health, Labor, and Welfare in collaboration with hospitals, clinics, and local pediatric practices in Japan between 1998 and 2002. The outcome for each patient was classified as either survival or death. Predictors of death were identified using logistic regression analysis.

Results: Four major prognostic factors for death were found to be significant by multivariate analysis ($P < 0.05$) in the 184 patients for whom we had complete data: elevation of aspartate aminotransferase, hyperglycemia, the presence of hematuria or proteinuria, and use of diclofenac sodium.

Conclusions: We identified patients who had factors associated with a poor prognosis, and these findings might be clinically useful for the management of this illness.

Key Words: influenza-associated encephalopathy, hypercytokinemia, diclofenac, IL-6, TNF-α

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Influenza-associated encephalopathy is a severe neurologic complication of influenza characterized by an abrupt onset of seizures and coma within a few days of developing a high fever.[1] The number of patients with influenza-associated encephalopathy in Japan has increased in recent years, with more than 100 children younger than 6 years of age dying annually from this severe disease.[1] Recently, reports of influenza-associated encephalopathy have also increased worldwide.[2,3]

Blood abnormalities, such as thrombocytopenia and elevated serum aspartate aminotransferase (AST), and brain computed tomography (CT) abnormalities are associated with poor outcome.[1] Cyclooxygenase inhibitors, particularly aspirin, are known to cause Reye syndrome.[4,5] In Japan, cyclooxygenase inhibitors such as diclofenac sodium and mefenamic acid, but not aspirin, are widely used as antipyretic drugs in children. We found that some nonsalicylate antipyretic drugs, including diclofenac sodium and mefenamic acid, may be associated with the development of influenza-associated encephalopathy or may affect the severity of the disease.[1] However, we were unable to thoroughly assess the relationship between the use of these medicines and prognosis.

The mortality rate of this disease is as high as 30% without treatment.[1] Therefore, for the administration of intensive care, it is important to identify the factors that affect its prognosis. We investigated 442 cases of influenza-associated encephalopathy reported from 1998 to 2002 in the Collaborative Study Group on Influenza-Associated Encephalopathy organized by the Japanese Ministry of Health, Labor, and Welfare, and analyzed the prognostic factors using multivariate logistic regression analysis. We report several factors related to the poor prognosis of influenza-associated encephalopathy. To our knowledge, this is the first nationwide study of the prognostic factors of influenza-associated encephalopathy.

METHODS

Study Design. Questionnaires were developed by the Collaborative Study Group on Influenza-Associated Encephalopathy to assess the number of cases in all hospitals, clinics, and local pediatric practices (total of 3500 sites) between 1998 and 2002. Subsequently, a second questionnaire was sent to each applicable facility. The second questionnaire requested information on age, sex, virus type, history, flu vaccination record, peak body temperature, symptoms, laboratory data, CT findings, medication, diagnostic methods of influenza virus infection, and disease outcome. The age, peak body temperature, and laboratory data were provided directly by participants, whereas the other data were gathered using a multiple-choice questionnaire. The flu vaccination record covered the season during which patients suffered from influenza-associated encephalopathy. The possible responses to the questions regarding vaccination history were "unknown," "no," "once," and "twice." Vaccination histories were not con-

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firmed by other methods. The laboratory data were obtained at admission. Recently, it has been reported that prognosis could be improved by therapies such as methylprednisolone pulse and hypothermia therapy.[6,7] However, it was not possible to obtain information regarding these therapies for this study.

The study protocol was approved by the Institutional Review Board of Nagoya University Hospital.

Case Definition. Influenza infection was defined on the basis of a positive result on viral culture, viral antigen testing, or viral ribonucleic acid polymerase chain reaction, or a 4-fold or greater rise in paired serum antibody titer test (hemagglutination inhibition or complement fixation test). Patients who did not meet all the criteria were excluded from the study. In viral antigen testing, the influenza type could not be determined in several cases because the diagnosis was made using a viral antigen test that could not distinguish between types A and B influenza. Patients were defined as having influenza-associated encephalopathy if they showed clinical symptoms and signs compatible with acute encephalopathy, such as altered consciousness (ie, delirium, confusion, and cognitive impairment) or loss of consciousness (ie, deep coma, coma, semicoma, stupor, and somnolence), and if these symptoms persisted for more than 24 hours. Patients with meningitis, myelitis, and febrile convulsions without prolonged unconsciousness were excluded. Cases of postictal unconsciousness with prompt recovery were classified as febrile convulsion. All of the cases reported as influenza-associated encephalopathy were reviewed thoroughly by members of the study group to confirm whether the diagnosis was appropriate. Doubtful cases were excluded from further analysis. The outcomes of influenza-associated encephalopathy were defined as survival or death. A survival outcome included all patients who were alive regardless of whether they had sequelae. In total, 442 influenza-associated encephalopathy cases in patients younger than 15 years of age were deemed appropriate for the study. Study participants provided informed consent or assented with parental consent.

Statistical Analysis. The data were analyzed using the Dr. SPSS software package version 2 (SPSS Inc. Tokyo, Japan). Twenty variables were analyzed to formulate a predictive model for death caused by influenza-associated encephalopathy. The variables identified as significant at $P < 0.05$ using univariate logistic regression analysis were entered into a multivariate logistic regression model, and the least significant variables were sequentially removed. In the multivariate logistic regression analysis, the model was adjusted by age, sex, virus type, history of allergy, record of flu vaccination, and use of acetaminophen. In the multivariate logistic regression analysis, a value of $P < 0.05$ was considered statistically significant. A P value between 0.05 and 0.20 was considered to show a tendency toward being a factor for poor prognosis because a risk existed of eliminating important prognostic factors in the logistic regression if the P values were restricted to <0.05. Odds ratios with 95% confidence intervals were also estimated.

RESULTS

Between 1998 and 2002, a total of 2624 sites contributed information and 693 patients were reported as potentially having influenza-associated encephalopathy, according to the primary questionnaire. In response to the second questionnaire, 585 cases from a total of 340 sites were reported and 442 cases in patients younger than 15 years of age deemed appropriate for further study. These included 97 patients who died and 345 patients who survived.

Of the 442 patients, 331 (74.9%) were between 1 and 6 years old, 232 were male, and 210 were female. No significant differences were observed in incidence or mortality between the sexes; 45 males and 52 females died. The death rates were 32.1%, 22.5%, 13.7%, and 16.4% in 1998–1999, 1999–2000, 2000–2001, and 2001–2002, respectively. We found 372 (84.2%) and 42 (9.5%) cases of type A and type B influenza, respectively. In the other 28 (6.3%) cases, the influenza type could not be determined. Fifty-four cases (22.1%) had a history of febrile convulsions.

Table 1 shows the numbers, percentages, odds ratios, and 95% confidence intervals from the univariate logistic regression analyses for the 20 variables divided by survival and death. In the univariate analyses, 14 variables had statistical significance ($P < 0.05$): peak body temperature of 40–41°C and ≥41°C; diarrhea; AST level of 100–500 IU/L and ≥500 IU/L; creatinine phosphokinase level of 200–1000 IU/L and ≥1000 IU/L; platelet count of $<10 \times 10^4/\mu L$; blood glucose level of <50 and ≥150 mg/dL; hematuria or proteinuria; CT showing edema, low-density areas, or hemorrhage; and use of diclofenac sodium and mefenamic acid for fever during influenza virus infection. These variables were retained for multivariate analysis.

The following variables related to patient background were also used in the multivariate analyses, although they were not significantly related to prognosis: age, sex, virus types, allergy history, flu vaccination record, and acetaminophen use. A history of febrile convulsion was excluded because of missing data.

We could not analyze all 442 patients in multivariate analysis because many of the factors with statistical significance in univariate analysis were missing data. A total of 13 of the 16 variables used in multivariate analysis had several missing data points. Thus, a total of 184 patients with complete data were included in multivariate analysis (Fig. 1). Reducing the number of cases from 442 to184 did not significantly alter the percentage of survival and death.

Table 2 summarizes the numbers and percentages of the 184 patients with complete data for these 16 variables and shows the adjusted odds ratios and 95% confidence intervals from multivariate logistic regression analysis. Four significant prognostic factors were used: AST ≥500 IU/L ($P = 0.04$), blood glucose ≥150 mg/dL ($P = 0.04$), hematuria or proteinuria ($P = 0.01$), and the use of diclofenac sodium ($P = 0.03$). The following variables showed a tendency toward being factors for poor prognosis: peak body temperature of 39–40°C ($P = 0.18$), 40–41°C ($P = 0.14$), and ≥41°C ($P = 0.05$); platelets $<10 \times 10^4/\mu L$ ($P = 0.18$); blood glucose <50 mg/dL ($P = 0.14$); CT showing edema, low-density areas, or hemorrhage ($P = 0.17$); and the use of mefenamic acid ($P = 0.09$).

Allergy history provided a nearly significant P value of 0.08 in the univariate analysis. The allergies reported included asthma, atopic dermatitis, and allergic rhinitis. We

TABLE 1. Characteristics on Admission and Univariate Analysis of Prognostic Factors in the 442 Patients Between 1998 and 2002

Variable	No. Patients		Odds Ratio (95% CI)	*P*
	Survival (n = 345)	Death (n = 97)		
Age group, yr				
<1	22	6	1	—
1–6	257	74	1.06 (0.41–2.70)	0.91
6–15	66	17	0.94 (0.33–2.69)	0.92
Sex				
Male	187	45	0.73 (0.47–1.15)	0.17
Female	158	52	1	—
Virus types				
Type A	294	78	0.59 (0.29–1.19)	0.14
Type B	29	13	1	—
Unclassified	22	6	—	—
Past history				
Allergy				
Yes	21	1	0.16 (0.02–1.21)	0.08
No	324	96	1	—
Febrile convulsion				
Yes	46	8	0.89 (0.38–2.07)	0.79
No	159	31	1	—
Flu vaccination record				
Yes	12	1	0.31 (0.04–2.37)	0.26
No	320	88	1	—
Peak body temperature, °C				
<39	45	4	1	—
39–40	107	21	2.21 (0.72–6.80)	0.17
40–41	125	35	**3.15 (1.06–9.36)**	**0.04**
≥41	18	18	**11.25 (3.34–37.86)**	**<0.001**
Symptoms				
Convulsion				
Yes	266	76	1.19 (0.66–2.15)	0.56
No	71	17	1	—
Abnormal behavior				
Yes	32	4	0.43 (0.15–1.25)	0.12
No	305	89	1	—
Arthralgia				
Yes	8	1	0.45 (0.06–3.62)	0.45
No	329	92	1	—
Diarrhea				
Yes	21	12	**2.23 (1.05–4.72)**	**0.04**
No	316	81	1	—
Blood examination AST, IU/L				
<100	227	22	1	—
100–500	53	28	**5.45 (2.89–10.27)**	**<0.001**
≥500	19	32	**17.38 (8.49–35.59)**	**<0.001**
Creatine phosphokinase, IU/L				
<200	189	33	1	—
200–1000	65	22	**1.94 (1.06–3.56)**	**0.03**
≥1000	16	20	**7.16 (3.37–15.22)**	**<0.001**
Platelet, $10^4/\mu L$				
<10	25	39	**10.79 (5.87–19.85)**	**<0.001**
≥10	256	37	1	—
Blood glucose, mg/dL				
<50	2	6	**34.71 (6.40–188.29)**	**<0.001**
50–150	162	14	1	—
≥150	105	57	**6.28 (3.33–11.84)**	**<0.001**
Urine test				
Normal	192	14	1	—
Hematuria or proteinuria	54	38	**9.65 (4.87–19.10)**	**<0.001**
Brain CT findings				
Normal	151	16	1	—
Edema, low density area, hemorrhage	138	62	**4.24 (2.34–7.70)**	**<0.001**
Medication				
Acetaminophen				
Yes	179	44	0.96 (0.59–1.58)	0.88
No	137	35	1	—
Diclofenac sodium				
Yes	19	15	**3.66 (1.77–7.59)**	**<0.001**
No	297	64	1	—
Mefenamic acid				
Yes	15	9	**2.58 (1.09–6.14)**	**0.03**
No	301	70	1	—

Total numbers for most variables are less than 442 because of incomplete answers to the questionnaire. Values in bold indicate statistically significant results. A logistic regression analysis was used to determine the significant predictors of death.

CI indicates confidence interval; AST, aspartate aminotransferase; CT, computed tomography.

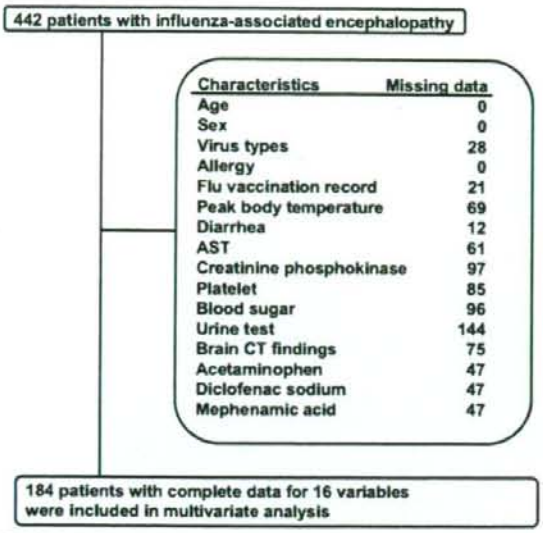

FIGURE 1. Flow chart of patient selection. A total of 442 patients with influenza-associated encephalopathy were reduced to 184 patients because of missing data points in the variables. Variables with missing data are shown with the number of missing data points. AST indicates aspartate aminotransferase; CT, computed tomography.

placed patients with allergies in the good prognosis group in univariate analysis. However, allergy history did not have a significant effect in multivariate analysis, possibly because of control of confounding.

DISCUSSION

An outbreak of encephalopathy suspected to have been caused by influenza infection prompted a national survey of influenza-associated encephalopathy at all hospitals and pediatric clinics in Japan, as well as this analysis of 442 cases. To our knowledge, this is the first study on the prognostic factors of influenza-associated encephalopathy. The mortality rate was as high as 30% without treatment.[1] Therefore, for the administration of intensive care, it is important to identify the factors that affect its prognosis. Using multivariate analysis, we identified several factors that were related to the poor prognosis of this disease.

A severely elevated transaminase level, thrombocytopenia, and hematuria or proteinuria were associated with an unfavorable outcome in influenza-associated encephalopathy. Although the pathogenesis of this disease is still unclear, several reports[8–10] have suggested that it involves cytokines, such as soluble tumor necrosis factor receptor-1, interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α), IL-8, and IL-10. Nerve and liver cells may be induced to undergo apoptosis in influenza-associated encephalopathy patients as a consequence of hypercytokinemia, resulting in disseminated intravascular coagulopathy and multiple organ failure.[8,10–13] Studies have reported hemophagocytosis in influenza-associated encephalopathy patients, suggesting the activation of macrophages and microglia cells by hypercytokinemia.[14] A severely elevated transaminase level, thrombocytopenia, and hematuria or proteinuria may be associated with disseminated intravascular coagulopathy, multiple organ failure, and hemophagocytosis resulting from hypercytokinemia induced by this disease.[8,10,11]

We also showed that hyperglycemia is a factor leading to poor prognosis. IL-6 may lead to increased cortisol levels, followed by a pronounced dose-dependent increase in blood glucose.[15,16] Therefore, we postulated that the systemic hypercytokinemia in influenza-associated encephalopathy causes hyperglycemia and that the glucose levels reflect the degree of pathogenicity.

Hypoglycemia provided a significant P value of <0.05 in univariate analysis. However, this did not result in a significant difference in the multivariate analysis, which was probably because the number of patients with hypoglycemia in multivariate analysis was reduced from 8 to only 2 cases, or 1.1% of patients, because of missing data. Hypoglycemia is a symptom of Reye syndrome with a very poor prognosis.[5,17,18] Medium-chain acyl-CoA dehydrogenase deficiency is the most common disorder of fatty acid β-oxidation, and occurs acutely in Reye's-like syndrome, which is often provoked by infection.[19] Reye's-like syndrome is similar to influenza-associated encephalopathy in several of its symptoms, such as loss of consciousness, seizures, and increased aminotransferase levels.[1,20] Therefore, we postulated that influenza-associated encephalopathy may include a metabolic disorder, such as medium-chain acyl-CoA dehydrogenase deficiency.

High-grade fever, particularly $\geq$41°C, showed a tendency toward being a prognostic factor in the multivariate analysis. Some patients with a poor outcome exhibit a mitochondrial β-oxidation disorder evoked by inactivated carnitine palmitoyltransferase II during high-grade fever in influenza-associated encephalopathy.[21] Analysis of the genotypes and allele compositions of carnitine palmitoyltransferase II have revealed a thermolabile phenotype that occurs more frequently in influenza-associated encephalopathy patients than in healthy subjects.[21] In addition, the use of the nonsalicylate antipyretic drug diclofenac to alleviate fever affected the prognosis of the disease, and the use of mefenamic acid tended to also influence the prognosis, whereas the use of acetaminophen was considered to have little effect. In May 2001, the Japanese Ministry of Health, Labor, and Welfare banned the use of these antipyretic drugs to alleviate fever in influenza infection based on the data of the Collaborative Study Group on Influenza-Associated Encephalopathy.[1] However, it is still unclear whether these drugs are related to the pathogenesis of influenza-associated encephalopathy. Shiga-like toxin II or Shiga-like toxin II-stimulated cytokines may change the brain penetration of diclofenac sodium and mefenamic acid, and consequently increase the risk of the drugs having central nervous system side effects.[22]

We did not find a significant correlation between the prognosis of influenza-associated encephalopathy and flu vaccination record. A more extensive study is required to reveal whether flu vaccination can improve the prognosis after developing the disease because only 13 patients, or 3.1% of the 442 cases, had been immunized against flu.

TABLE 2. Characteristics of Admission and Multivariate Analysis of Prognostic Factors in the 184 Patients Included in This Study Between 1998 and 2002

Variable	No. Patients		Odds Ratio (95% CI)	*P*
	Survival (n = 149)	Death (n = 35)		
Age, yr				
<1	11	1	1	—
1–6	115	31	1.12 (0.05–26.46)	0.94
6–15	23	3	0.09 (0.001–6.24)	0.26
Sex				
Male	76	12	0.49 (0.14–1.74)	0.27
Female	73	23	1	—
Virus types				
Type A	135	29	0.56 (0.06–5.78)	0.63
Type B	14	6	1	—
Past history				
Allergy				
Yes	8	1	1.19 (0.06–24.19)	0.91
No	141	34	1	—
Flu vaccination record				
Yes	7	1	0.29 (0.02–5.57)	0.41
No	142	34	1	—
Peak body temperature, °C				
<39	22	1	1	—
39–40	59	9	11.72 (0.31–442.39)	0.18
40–41	60	18	15.65 (0.42–577.88)	0.14
≥41	8	7	42.61 (0.98–1851.87)	0.05
Symptom				
Diarrhea				
Yes	9	5	2.28 (0.26–20.25)	0.46
No	140	30	1	—
Blood examination AST, IU/L				
<100	103	7	1	—
100–500	33	8	1.76 (0.40–7.64)	0.45
≥500	13	20	**7.88 (1.15–54.00)**	**0.04**
Creatine phosphokinase, IU/L				
<200	101	15	1	—
200–1000	37	9	1.03 (0.20–5.30)	0.97
≥1000	11	11	1.08 (0.13–9.16)	0.94
Platelet, $\times 10^4/\mu L$				
<10	16	20	3.79 (0.55–26.24)	0.18
≥10	133	15	1	—
Blood glucose, mg/dL				
<50	1	1	28.79 (0.35–2372.98)	0.14
50–150	83	5	1	—
≥150	65	29	**4.73 (1.10–20.30)**	**0.04**
Urine test				
Normal	108	10	1	—
Hematuria or proteinuria	41	25	**7.96 (1.76–35.92)**	**0.01**
Brain CT findings				
Normal	85	6	1	—
Edema, low density area, hemorrhage	64	29	2.59 (0.68–9.90)	0.17
Medication				
Acetaminophen				
Yes	94	21	1.46 (0.38–5.56)	0.58
No	55	14	1	—
Diclofenac sodium				
Yes	8	7	**16.34 (1.27–210.18)**	**0.03**
No	141	28	1	—
Mefenamic acid				
Yes	4	5	9.44 (0.70–127.73)	0.09
No	145	30	1	—

Values in bold indicate statistically significant results. A logistic regression analysis was used to determine the significant predictors of death.

CI indicates confidence interval; AST, aspartate aminotransferase; CT, computed tomography.

The survey of influenza-associated encephalopathy is continuing. However, some parts of the questionnaires have been changed and fewer sites are now included in the survey. These changes were made mainly because the "Private Information Protection Law" came into effect in Japan in 2003, making it very difficult to obtain individual information. We did not obtain information regarding the therapy for influenza-associated encephalopathy because there were no standardized therapeutic protocols between 1998 and 2002. Recently, it has been reported that certain therapeutic regimens can improve the prognosis of influenza-associated encephalopathy.[6,7] In 2005, therapies such as methylprednisolone pulse, plasma exchange,

and hypothermia therapy were proposed by the Collaborative Study Group on Influenza-Associated Encephalopathy, and further studies to improve influenza-associated encephalopathy prognosis via therapy are currently underway.

In conclusion, we identified several factors related to the poor prognosis of influenza-associated encephalopathy. Use of diclofenac sodium was the causal factor of poor prognosis. The other factors seem to reflect systemic hypercytokinemia, which is thought to play a role in the pathogenesis of the disease. However, all of these factors (with the exception of the use of diclofenac sodium) may be secondary to the disease process because they are seen in subjects who are moribund from a number of causes. Although these factors cannot be used to make an early diagnosis, our results have 2 major implications: the prognostic factors that we identified are easy to examine clinically, and these factors are important for the administration of intensive care in cases of influenza-associated encephalopathy.

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Case report

Transient subacute cerebellar ataxia in a patient with Lambert-Eaton myasthenic syndrome after intracranial aneurysm surgery

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Abstract

Several reports have presented patients with subacute cerebellar ataxia (CA) and Lambert-Eaton myasthenic syndrome (LEMS). Some clinical features of those patients have been described in the previous reports, manifestation of subacute CA prior to LEMS or a co-existence of both diseases, a high incidence of malignancy, and less efficacy of the treatment for subacute CA compared with that for LEMS. Cerebellar ataxia in some patients with LEMS has been suggested to be caused by antibodies to P/Q-type voltage-gated calcium channels (VGCCs). We report herein a patient with subacute CA and LEMS. Cerebellar ataxia appeared 15 months after the occurrence of LEMS, and the onset of CA was thought to be due to serum anti-P/Q-type VGCC antibodies. The clinical course of this patient was atypical, as follows: (1) LEMS preceded subacute CA, which developed after intracranial aneurysm surgery, (2) no malignancy was detected when both diseases co-existed, (3) symptoms of LEMS did not progress with the onset of CA, and (4) there was a definite improvement in symptoms of CA and ^{123}I-IMP SPECT imaging findings after steroid administration. In addition, it is remarkable that LEMS became aggravated in electrophysiologic examinations, in contrast to subacute CA. We suggest that these atypical features of subacute CA and the changes in LEMS may be associated with a balance between the amount of serum anti-P/Q-type VGCC antibodies and the susceptibility of the cerebellum and presynaptic nerve terminals to the antibodies. More cases are needed to investigate the mechanisms involved. The subacute CA and LEMS in this patient have remained comparatively silent after the withdrawal of steroids, and we are continuing to observe her condition.

Keywords: Lambert-Eaton myasthenic syndrome; LEMS; Cerebellar ataxia; P/Q-type voltage-gated calcium channels; VGCC; Steroid; Intracranial aneurysm surgery

1. Introduction

Lambert-Eaton myasthenic syndrome (LEMS) and subacute cerebellar ataxia (CA) consist of paraneoplastic neurologic syndromes. Occasionally, they have been reported to co-exist in individual patients. These patients have a high frequency of cancers and subacute CA is thought to be caused by autoantibodies against antigens co-expressed by the cancers and by neurons associated with LEMS. For these patients, it is considered most effective to treat the cancers. In addition, 3,4-diaminopyridine (3,4-DAP), steroids, or immunosuppressants are often administered, and plasmapheresis is attempted. Subacute CA has been reported to be less responsive to these treatments than LEMS.

We encountered a patient with the onset of LEMS at 62 years of age, followed by subacute CA 15 months later. Subacute CA had developed 4 months after intracranial aneurysm surgery. No malignancies were observed when subacute CA occurred. Steroid administration greatly improved the cerebellar ataxia. Since our patient had several atypical features

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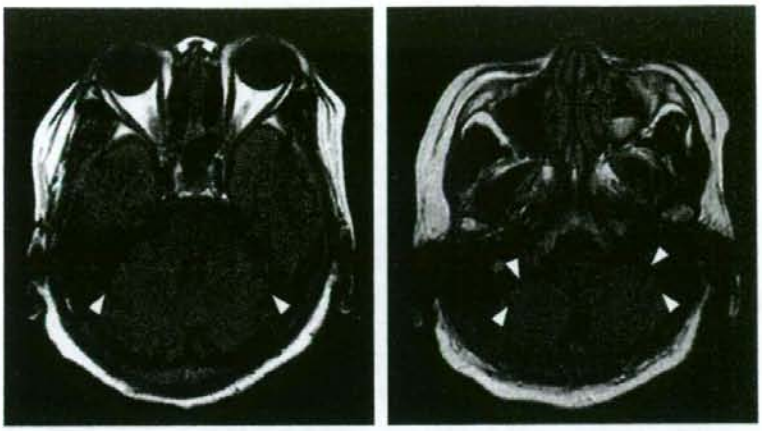

Fig. 1. Brain MRI (1.5T FLAIR) of our patient. Symmetric dilation of cerebellar fissure (arrow heads), indicating mild cerebellar atrophy.

compared with reported cases, we present our case herein and detail the clinical course.

2. Case report

A 62-year-old woman developed weakness in the distal portion of her lower limbs. This symptom slowly progressed to the proximal portion until she required a walker to assist herself while ambulating. Proximal weakness in the upper limbs followed after 4 months. She was admitted to our hospital for evaluation. Her neurologic examination demonstrated diplopia on leftward gaze, weakness of four limbs with proximal dominance, diminished deep tendon reflexes of four limbs, post-exercise potentiation of the right biceps brachii tendon reflex, and a waddling gait with assistance. Repetitive nerve stimulation (RNS) test involving stimulation of the left ulnar nerve and detection with surface electrodes overlying the left abductor digiti minimi (ADM) showed low amplitude of the distal CMAP (0.69 mV) and marked waxing (>500%) at 100 Hz repetitive stimulations. The serum titer of anti-P/Q-type voltage-gated calcium channels (VGCCs) antibodies was 59.3 pmol/L (cutoff value, <20 pmol/L). Therefore, she was diagnosed with LEMS. A systemic investigation detected no malignancies. 3,4-Diaminopyridine was prescribed after approval by the Ethics Committee of Hokkaido University Graduate School of Medicine. It was administered at 80 mg/day for a few months with pyridostigmine bromide (180 mg/day). The weakness slowly improved and she was able to walk with a walker after 1 month. After 6 months, she developed an aneurysm at the M2 segment of the right middle cerebral artery, which was clipped. After emerging from general anesthesia (sevoflurane, fentanyl, prppofol, and vecronium bromide), her weakness worsened. However, the 3,4-DAP and pyridostigmine bromide improved the symptoms to the pre-operative state without increments, and she was discharged 2 months later.

Two months following discharge, she developed dysarthria, diplopia, clumsiness of the four limbs, and truncal instability. Communicating with others, writing, and walking with support had become difficult over a period of 2 weeks. Therefore, she was readmitted to our hospital. On the neurologic examination, slurred speech, gaze nystagmus, dysmetria of the four limbs, and severe truncal ataxia were observed. The hyporeflexia and mild proximal weakness involving the four limbs were unchanged. RNS test of the left ulnar nerve measured on the left ADM demonstrated a greater distal CMAP (2.7 mV) and milder waxing (136%) at 100 Hz repetitive stimulations than observed before treatment during the first admission. Autoantibodies associated with autoimmune systemic vasculitis (anti-nuclear, anti-DNA, anti-Sm, anti-RNP, anti-SSA, anti-SSB, anti-GAD, and anti-gliadin antibodies) were negative. The anti-thyroglobulin and anti-thyroperoxidase antibodies titers were high (5.22 U/ml [normal range, <0.15 U/ml] and 23.92 U/ml [normal range, <0.15 U/ml], respectively). However, anti-α-enolase antibody, which is frequently found in patients with Hashimoto encephalopathy, was not detected. Serum titers of onconeuronal antibodies (anti-Yo, anti-Ri, anti-Hu, anti-CV-2, anti-Tr, anti-Ma-2, and anti-amphiphysin antibodies) were negative, but the anti-P/Q-type VGCC antibody titer demonstrated a mild elevation (128.6 pmol/L) compared with that observed during the first examination. Serum tumor markers (CEA, CA19-9, SCC, Pro-GRP, NSE, NCC-ST-439, CA15-3, CA125, and soluble IL-2 receptor) were within normal ranges. Examination of cerebrospinal fluid (CSF) disclosed a slight rise in the IgG index (0.80 [normal range, >0.70]) without pleocytosis (2/μl), elevation of protein (42 mg/dl), or a decrement in glucose (59 mg/dl). In the CSF, a significant rise of titers of anti-viral antibodies (HSV, VZV, EBV, and CMV), malignant cells, and anti-P/Q-type VGCC antibodies were not detected. A systemic imaging study (neck, chest, abdominal, and pelvic CT; gastrofiberoscopy; colonofiberoscopy; mammography;

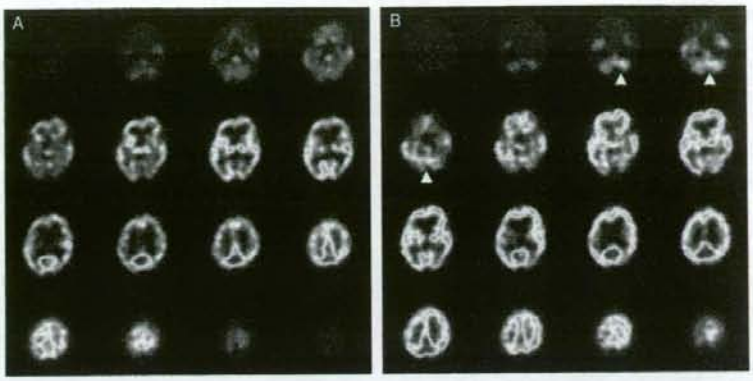

Fig. 2. ^{123}I-IMP SPECT of our patient. (A) Before steroid treatment (May, 2006). (B) After steroid treatment (July, 2006). ^{123}I-IMP SPECT shows hypoperfusion of bilateral cerebellar hemispheres when subacute cerebellar ataxia occurred (A). One month after the beginning of the administration of steroids, cerebellar blood flow was increased (B, arrow heads).

and FDG-PET) demonstrated no evidence of cancer. A brain MRI showed no significant abnormalities, except a slight bilateral cerebellar atrophy (Fig. 1). ^{123}I-IMP SPECT depicted marked hypoperfusion of the bilateral cerebellar hemispheres (Fig. 2A).

We administered 1 g of methylprednisolone intravenously for 3 days. Afterward, we prescribed 60 mg/day (1 mg/kg) of oral prednisolone and tapered the dosage 10 mg/day every 2 weeks, with discontinuation by 12 weeks. This treatment improved the slurred speech, nystagmus, and limb and truncal ataxias. Her condition was quantified to be 53–39 by the International Cooperative Ataxia Rating Scale (ICARS) and 50–65 by the Barthel Index (BI) during these 12 weeks. Her speech and writing became recognizable, and she was able to walk with a walker. While the slight cerebellar atrophy remained on the MRI, ^{123}I-IMP SPECT showed a better cerebellar blood flow (Fig. 2B). Approximately 12 weeks after beginning steroid administration, no cancers were detected by systemic CT and FDG-PET. RNS test on the ADM by stimulating the left ulnar nerve showed a decrease in the distal CMAP (0.6 mV) and an increment of waxing (385%) at 100 Hz repetitive stimulations at 4 weeks after the beginning of treatment. However, weakness of the neck and limbs were unchanged through the course of treatment. She has maintained this ADL without exacerbation of the cerebellar ataxia and weakness after withdrawal of steroids.

3. Discussion

In this case, subacute CA occurred 15 months after the onset of LEMS and developed 4 months after intracranial aneurysm surgery. The likelihood that the etiology of subacute CA was direct tumor invasion of the cerebellum, or a degenerative, cerebrovascular, infectious, or systemic autoimmune inflammatory disease was small from the results of our comprehensive examinations. Cerebellar ataxia associated with anti-α-enolase, anti-gliadin, or anti-GAD antibodies was also ruled out. We considered that the cause of subacute CA was analogous with that of paraneoplastic cerebellar ataxia (PCA). Paraneoplastic cerebellar ataxia results from an autoimmune mechanism that is mediated by autoantibodies, resulting in cerebellum damage. While no serum onconeuronal antibodies were detected in our case, we suggest that subacute CA was caused by serum anti-P/Q-type VGCC antibodies, as shown previously [1].

According to previous reports, patients with co-existing subacute CA and LEMS tend to have progressively worsening CA or to initially present symptoms of both diseases. There have been only two reported cases of patients with LEMS that preceded subacute CA [2,3]. Thus, our patient was a rare case. Although the interval between the onset of both diseases seemed longer in our case than previously reported, to verify this fact, examinations of similar cases are needed.

Our patient showed a mild rise in the serum anti-P/Q-type VGCC antibody titer on the second admission, but these antibodies were not detected in the CSF. Therefore, we suggest that the production of anti-P/Q-type VGCC antibodies and rise in titer may be a trigger for the occurrence of subacute CA in patients with LEMS. The detection of these antibodies in CSF has been reported in only 20–40% of cases in which investigations of CSF were conducted [1,2,4]. Therefore, the CSF titer seems of little diagnostic significance at present. However, CA in our case developed after intracranial aneurysm surgery. This fact may indicate that the operation influenced the function of the blood-brain barrier (BBB) and promoted circulating anti-P/Q-type VGCC antibodies to pass through the BBB, although CSF anti-P/Q-type VGCC antibodies a few weeks after a neurosurgical procedure could not be measured. Cerebellitis is ruled out by an absence of CSF pleocytosis and Hashimoto encephalopathy, by absence of anti-alpha enolase antibody.

In most cases of subacute CA with LEMS, cancers are detected, of which small cell lung cancer (SCLC) is the most

common [1,3–8]. However, our patient had no clinical evidence of malignancy when examined after presenting with subacute CA and 3 months after steroid administration was initiated. These observations indicate another autoimmune mechanism that produced anti-P/Q-type VGCC antibodies, other than that based on molecular mimicry between P/Q-type VGCC and antigens expressed on the surface of cancer cells.

In previous reports of subacute CA-LEMS, subacute CA has shown little response to various treatments [1,2,5,7]. Paraneoplastic cerebellar ataxia without LEMS is also considered unlikely to respond to treatment [9]. However, the CA in our patient was definitely improved by steroid administration, as quantitatively demonstrated through ICARS and BI. In addition, ^{123}I-IMP SPECT revealed an increase of blood flow in the cerebellum after treatment (Fig. 2B). The histologic cerebellar damage may have remained mild in our case, although 2 months passed after the onset of subacute CA. Two months is a comparatively long time for PCA, which is considered to cause irreversible damage in the early stages [5]. One of the factors associated with the extent of cerebellar damage may be the balance between the amount of anti-P/Q-type VGCC antibodies in serum and the susceptibility of cerebellar tissue to these antibodies. However, the characteristics of susceptibility are unknown.

In our case, LEMS did not worsen, as assessed by the physical and electrophysiologic examination, in spite of a rise in the serum titer of the anti-P/Q-type VGCC antibodies with the onset of subacute CA. This discrepancy may also be associated with the susceptibility of P/Q type VGCCs at presynaptic nerve terminals to the antibodies. While subacute CA was improved by steroid administration after 1 month of treatment, LEMS showed subclinical deterioration in electrophysiologic tests. This observation was also difficult to explain. It is rare that only the CA improve by treatment in a subacute CA-LEMS patient [6,8]. In order to reveal the causes of these phenomena, we need to examine the serum titer of anti-P/Q-type VGCC antibodies after steroid administration in additional cases.

4. Conclusion

The clinical course of our patient was atypical as follows: (1) LEMS preceded subacute CA, which developed after intracranial aneurysm surgery, (2) no malignancy was detected when both diseases co-existed, (3) symptoms of LEMS did not progress with the onset of CA, and (4) CA showed a definite improvement in symptoms and by ^{123}I-IMP SPECT imaging after steroid administration. Our case indicates that there may be more cancer-free subacute CA-LEMS patients with treatable CA than expected. In addition, the clinical features of our case includes a number of suggestions about the treatability of CA, the molecular mechanisms involved, and a treatment regimen with no concern for the occurrence of malignancies. It is important that similar cases will be described in the future.

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Case Report

A patient with anti-aquaporin 4 antibody who presented with recurrent hypersomnia, reduced orexin (hypocretin) level, and symmetrical hypothalamic lesions

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Abstract

Recent studies have demonstrated that hypothalamic lesions associated with brain tumor, head trauma, and encephalopathy can cause symptomatic hypersomnia with a reduced orexin (hypocretin) level in the cerebrospinal fluid (CSF). Aquaporin 4 (AQP4), a member of the AQP superfamily, is strongly expressed in the hypothalamus in which orexin (hypocretin)-containing neurons are primarily concentrated. We report the case of a patient with a serum anti-AQP4 antibody who presented with recurrent hypersomnia, symmetrical hypothalamic lesions with long spinal cord lesions on MRI, and a reduced CSF orexin (hypocretin) level, all of which were improved simultaneously by steroid therapy. Further studies should be performed to determine the roles of anti-AQP4 antibody positivity in patients with hypersomnia associated with orexin (hypocretin) deficiency and hypothalamic lesions.

Keywords: Hypersomnia; Hypothalamus; Aquaporin 4; Orexin (hypocretin)

1. Introduction

Aquaporin 4 (AQP4), a member of the AQP superfamily, is strongly expressed in the hypothalamus [1] in which orexin (hypocretin)-containing neurons are primarily concentrated [2]. Recently, the NMO-IgG/anti-AQP4 antibody, which can be detected in the serum of patients with neuromyelitis optica (NMO) and an opticospinal form of multiple sclerosis (OSMS), has been shown to selectively bind to AQP4. [3,4] Herein, we provide the first case report of a patient with the anti-AQP4 antibody who presented with recurrent hypersomnia as the main symptom and a reduced orexin (hypocretin) level in the cerebrospinal fluid (CSF).

2. Case

A 42-year-old Japanese woman developed acute-onset hypersomnia within several days with no apparent causes or triggers. She slept for more than 16 h per day and exhibited excessive daytime sleepiness (Epworth sleepiness score, ESS = 19/24). She experienced no

Abbreviations: AQP, aquaporin; NMO, neuromyelitis optica; ESS, Epworth sleepiness score; CSF, cerebrospinal fluid.

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symptoms suggestive of cataplexy, sleep paralysis or hypnagogic hallucinations. Her hypersomnia and excessive daytime sleepiness persisted for one month and disappeared spontaneously. At the age of 43, she suffered from acute-onset hypersomnia again with a three-months history of sensory disturbance of her limbs and was admitted to our hospital for the evaluation of her symptoms.

Neurological examinations revealed hypersomnia (about 14 h of sleep per day), excessive daytime sleepiness (ESS = 13/24), and transverse myelopathy at the cervical level. No optic nerve involvement could be detected by ophthalmologic examination or by analyses using visual evoked potential. CSF analysis revealed mild pleocytosis (24/mm^3) and an elevated IgG index of 0.77. The level of CSF orexin (hypocretin), a hypothalamic neuropeptide that regulates arousal and sleep, mildly decreased (191.2 pg/ml; normal range 290 ± 65 pg/ml) [5]. A brain MRI revealed bilateral symmetrical hypothalamic and periaqueductal lesions (Fig. 1A–B) and a spinal MRI revealed an extensively longitudinal spinal cord lesion (Fig. 1C–D).

She was administered intravenous methylprednisolone (1000 mg/day) for three successive days, followed by oral prednisolone at a dose of 60 mg, which was then tapered. Her hypersomnia and excessive daytime sleepiness gradually improved. Two months after the steroid administration, she recovered completely from her sleep disturbance, and slightly from her sensory disturbance. After the treatment, her CSF orexin (hypocretin) level increased to 291.7 pg/ml, and her CSF-IgG index and CSF cell count normalized. For measurement of orexin (hypocretin), all CSF samples were assayed in the same batch to minimize interassay variability. Symmetrical hypothalamic lesions on the MRI became undetectable and the size of the spinal cord lesions was reduced. Her serum taken on admission was tested for the anti-AQP4 antibody [4] and NMO-IgG [6] by the indirect immunofluorescence methods, and turned out to be positive.

3. Discussion

We encountered a patient who presented with recurrent hypersomnia, symmetrical hypothalamic lesions

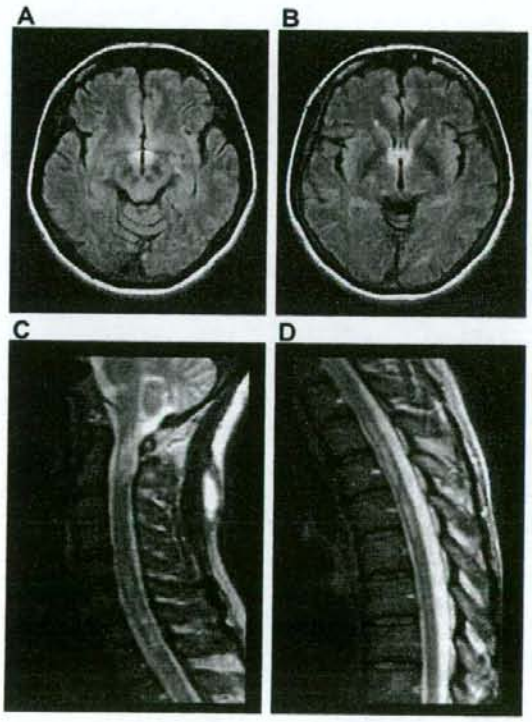

Fig. 1. Brain axial fluid attenuation inversion recovery magnetic resonance images show hyperintensity areas involving bilateral symmetrical hypothalami (A) and periaqueductal lesions (B). Spinal sagittal T2-weighted magnetic resonance images show hyperintensity areas of the cervicothoracic cord from the C6 level to the Th7 level and of the cervical cord at the C2 level (C) with mild swelling (C and D).

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and long spinal cord lesions on her MRI, and a reduced CSF orexin (hypocretin) level, all of which were improved simultaneously by steroid therapy. Our final diagnosis of this patient is high-risk syndrome of NMO defined as myelitis with ⩾3 vertebral segment spinal cord lesions [7]. She did not satisfy the revised NMO diagnostic criteria [8] owing to the lack of evidence of optic neuritis.

Recent studies have demonstrated that hypothalamic lesions associated with brain tumor, head trauma, and encephalopathy can cause symptomatic hypersomnia with a reduced CSF orexin (hypocretin) level [2]. We considered that her hypersomnia might have been caused by hypothalamic lesions, because orexin (hypocretin) neurons, which regulate arousal and sleep, are concentrated in the lateral hypothalamus [2]. In this patient, only mild orexin (hypocretin) deficiency existed while the subject expressed hypersomnolence. We speculate that the hypothalamic lesions might cause a dysfunction in the central histaminergic neurons localized in the tuberomammillary nucleus of the posterior hypothalamus, which also have an important role in wakefulness, in addition to the orexin (hypocretin) neurons [9]; nonetheless, further study is required to confirm this speculation.

AQP4 is expressed throughout the central nervous system, especially in periaqueductal and periventicular regions involving the hypothalamus of the brain [1,10] and is found in non-neuronal structures, such as astrocytes and ependymocytes, but is absent from neurons [1]. Hypothalamic lesions are observed in 3 of 89 NMO patients and 31 high-risk syndrome of NMO patients seropositive for NMO-IgG; however, no patients presenting hypersomnia or other sleep disturbances were described [10]. Poppe et al. have reported the cases of two NMO patients presenting excessive somnolence with symmetrical hypothalamic lesions [11], although they measured neither NMO-IgG nor anti-AQP4 antibody nor CSF orexin (hypocretin) level. This is the first report of a patient presenting symptomatic hypersomnia with symmetrical hypothalamic lesions associated with anti-AQP4 antibody positivity and a reduced CSF orexin (hypocretin) level. Taken together, these findings suggest that the anti-AQP4 antibody may have a role in her hypothalamic lesions, although it still remains undetermined how the anti-AQP4 antibody can cause hypothalamic lesions.

From our experience, we consider that patients with the anti-AQP4 antibody might develop hypersomnia, which can be improved by steroid therapy. Further studies should be performed to determine the roles of anti-AQP4 antibody positivity in patients with hypersomnia associated with orexin (hypocretin) deficiency and hypothalamic lesions.

Funding/support

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Salivary Chromogranin A: Useful and Quantitative Biochemical Marker of Affective State in Patients with Amyotrophic Lateral Sclerosis

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Abstract

Objective To evaluate the affective state biochemically and quantitatively in amyotrophic lateral sclerosis (ALS) patients using salivary chromogranin A (CgA) measurement.

Subjects and Methods Twelve moderate and 12 terminal ALS patients defined using the ALS Health State Scale were studied. The correlation between salivary CgA levels and the 40-item ALS assessment questionnaire (ALSAQ-40) scores was investigated in 12 moderate ALS patients. Moreover, salivary CgA levels in 12 terminal ALS patients, in whom the emotional functioning score could not be assessed, were compared with those in 12 moderate ALS patients, 7 patients with tube-fed vascular dementia, and in 26 healthy volunteers.

Results There were individual differences in salivary CgA levels in spite of similar severity of disease; however, mean salivary CgA levels in terminal ALS patients, in whom the emotional functioning score based on interview could not be assessed, was significantly higher (12.58±2.79 pmol/mL) than in patients with moderate ALS (6.36±1.62 pmol/mL, $p<0.05$), tube-fed vascular dementia (4.04±2.04 pmol/mL, $p<0.01$), and healthy volunteers (3.77±1.90 pmol/mL, $p<0.01$). Moreover, a statistically significant positive correlation was observed between salivary CgA levels and emotional functioning scores on ALSAQ-40 in moderate patients ($r=0.892$, $p<0.01$).

Conclusion Salivary CgA may be a useful and quantitative biochemical marker of the affective state, not only in moderate, but also in terminal ALS. Periodic salivary CgA measurements over the long term and/or in various situations could have therapeutic implications for the quality of life of these patients.

Key words: amyotrophic lateral sclerosis, salivary chromogranin A, 40-item ALS assessment questionnaire, affective state, quality of life

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Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease with no known effective treatment or cure. Hopelessness and end-of-life concerns are often serious complaints in patients with ALS, and they often become depressed during their clinical course (1, 2). Simmons et al reported that quality of life (QOL), as assessed by ALS patients, does not correlate with measures of strength and physical function, but appears to depend on psychological and existential factors (3); therefore, an important parameter when considering the treatment of these patients is their quality of emotional function. The 40-item ALS assessment questionnaire (ALSAQ-40), which includes an emotional functioning score, is one of the most widely

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validated measures of health status in these patients (4-6); however, as inevitably found with questionnaire-based outcome instruments, some data will be missing (7). In addition, this rating scale cannot assess the emotional function in advanced ALS patients because it is based on medical examination by interview. Useful biochemical markers of the affective state in advanced patients have not yet been developed.

Recently, attention has been focused on chromogranin A (CgA) as an endocrinological stress marker, because this molecule, which is co-released with catecholamine, is a better index of sympathetic activity (8, 9). CgA is a soluble protein and its concentration can be measured in saliva (10, 11). As symptoms of disturbed autonomic function are rare in ALS, the salivary CgA level may be an index of affective state in these patients; however, the association of these levels with the affective state has not previously been investigated in ALS.

The aim of the study was to evaluate if salivary CgA could be a useful and quantitative marker of the affective state in ALS, especially in advanced patients in whom the emotional functioning score based on medical examination by interview cannot be assessed.

Subjects and Methods

Subjects

Twenty-four patients (11 men and 13 women, mean age 69.3±5.6 years; 12 moderate and 12 terminal patients) with a diagnosis of ALS, who had been respectively evaluated at the Department of Neurology, Nishibeppu National Hospital, Japan from 1998 to 2005 or at the Division of Neurology and Neuromuscular Disorders, Department of Brain and Nerve Science, Oita University Faculty of Medicine, Japan from 2003 to 2005 were available for the study. According to the WFN El Escorial criteria (Airlie House, Warrenton, Virginia, April 2-4, 1998), all patients were clinically definitive for ALS, and their severity levels were defined using the ALS Health State Scale (12). Moderate ALS is defined as mild deficit in all 3 regions (upper motor neurons, lower motor neurons, and brainstem) or moderate to severe deficit in 1 region, while the other 2 regions are normal or mildly affected. Terminal ALS is defined as nonfunctional use of at least 2 regions and moderate or nonfunctional use of the third region. The patients with depression diagnosed by their clinical histories were not included in our study. Six out of 12 moderate ALS patients complained of respiratory difficulty, but the other 6 moderate patients were free from such complaint. Twelve terminal ALS patients were all under invasive mechanical ventilation. Before this study, all of the moderate ALS patients took meals periorally, and all 12 terminal ALS patients were percutaneous endoscopic gastrostomy (PEG)-tube fed.

In addition, 7 patients with PEG-tube fed vascular dementia (4 men and 3 women, mean age 72.3±6.7 years), and 26 age-matched healthy volunteers (13 men and 13 women, mean age 69.1±4.6 years) were also included. According to the NINDS-AIREN (13), all 7 patients with vascular dementia were clinically "probable", and all these patients did not show the symptoms of autonomic dysfunction and abnormalities of short-term heart rate variability. All subjects were nonsmokers. Approval for the study was obtained from the ethical committees of Oita University and Nishibeppu National Hospital. The purpose and procedure of the experiment were explained thoroughly, and consent was obtained from all subjects.

Salivary sampling and salivary CgA measurement

All 12 moderate patients who usually took meals periorally were studied after more than 12 hours fast because chewing stress affects the secretion of salivary CgA (14). As the half-life of salivary CgA is only 70 minutes (8), the influence of chewing stress on salivary CgA secretion could be sufficiently avoided in this study. On the other hand, all 12 terminal patients and 7 patients with vascular dementia who did not have the chewing stress (PEG-tube fed) were also studied after more than 12 hours of fasting. Thus, all patients were assessed under the similar feeding conditions in this study. Moreover, to avoid variations due to circadian rhythm (15), saliva samples were collected at a fixed time (10:00 am) and were frozen immediately. The samples were stored at -80℃ until analysis. The concentrations of salivary CgA were measured using a YK070 chromogranin A EIA kit (Yanaihara Institute Inc., Shizuoka, Japan) (10, 11). No drugs with any influence on the secretion of salivary CgA were administered during this study, and none of the patients or healthy volunteers showed any symptoms of disturbed autonomic function.

Questionnaire survey

The 12 moderate ALS patients were asked to answer the ALSAQ-40 prior to the experiment. The ALSAQ-40 questions are designed to measure five dimensions of health status: physical mobility, activities of daily living (ADL), eating and drinking, communication, and emotional functioning. The dimension scores are coded on a scale of 0 (perfect health as assessed by this measure) to 100 (worst health as assessed by this measure) (4-6). This survey was administered by conducting in-depth interviews with patients.

Statistics

Comparisons within multiple groups were performed using two-way analysis of variance followed by Duncan's multiple range tests. In all procedures, $p<0.05$ was considered significant. Values are expressed as the mean ± SD. Correlations between the salivary CgA level and each dimension score of ALSAQ-40 were investigated by Spearman's nonparametric correlation coefficient by rank.

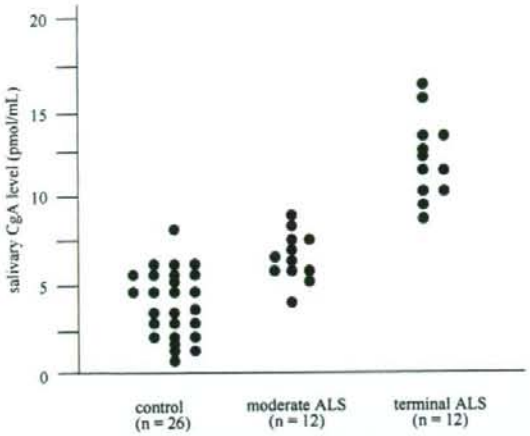

Figure 1. Salivary CgA levels in all subjects.

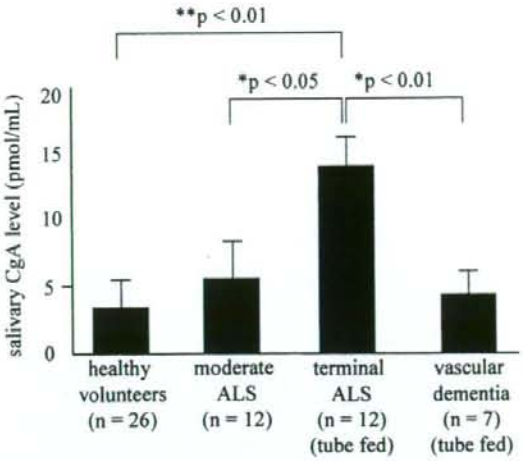

Figure 2. Comparison of salivary CgA levels in terminal ALS patients, moderate ALS patients, PEG-tube fed vascular dementia patients, and healthy volunteers. Values are expressed as the mean ± SD. *Mean salivary CgA level in terminal ALS patients was significantly higher than that of moderate ALS patients ($p<0.05$). *Mean salivary CgA level in terminal ALS patients was significantly higher than that of healthy volunteers ($p<0.01$). *Mean salivary CgA level in terminal ALS patients was significantly higher than that of tube-fed vascular dementia patients ($p<0.01$).

Results

1. Salivary CgA levels

Salivary CgA levels in all subjects are shown in Fig. 1. There were some individual differences in salivary CgA levels even in the same group; however, the mean salivary CgA level in terminal ALS patients, in whom the emotional functioning score based on medical examination by interview could not be assessed, was significantly higher (12.58±2.33 pmol/mL) than in moderate ALS patients (6.36±1.62 pmol/mL, $p<0.05$), tube-fed vascular dementia (4.04±2.04 pmol/mL, $p<0.01$), and healthy volunteers (3.77±1.90 pmol/mL, $p<0.01$), as shown in Fig. 2. Gender was not related to salivary CgA levels in all groups.

2. Correlation between salivary CgA levels and the scores on ALSAQ-40 in moderate ALS patients

Descriptive statistics for respondents on the 5 dimensions of the ALSAQ-40 in moderate ALS patients are shown in Table 1. The mean emotional functioning score was 37.50± 22.61 in these patients. There were some individual differences in these dimension scores on ALSAQ-40 even in a similar severity of disease, but gender was not related to scores in this group.

Although no significant correlation was observed between the salivary CgA level and physical mobility ($r=0.412$, $p>0.1$), ADL ($r=0.399$, $p>0.1$), eating and drinking ($r=0.550$, $p>0.05$), and communication scores ($r=0.544$, $p>0.05$) on ALSAQ-40 (Fig. 3A-D), a statistically significant highly positive correlation was observed between salivary CgA level and the score on emotional functioning in these patients ($r=0.892$, $p<0.01$, Fig. 3E).

Table 1. Descriptive Statistics for Respondents in 5 Dimensions of the ALSAQ-40 in Moderate ALS Patients

Dimension	Mean (SD)	Min–Max
Physical mobility	30.00 (17.06)	0–60
ADL	37.50 (16.03)	10–60
Eating and drinking	41.67 (19.92)	10–80
Communication	31.67 (14.03)	10–70
Emotional function	37.50 (27.61)	0–80

Discussion

To our knowledge, this is the first investigation of the relationship between the salivary CgA level and affective state in ALS patients. In this study, a statistically significant highly positive correlation was observed only between the salivary CgA level and the score on emotional functioning in ALSAQ-40 in moderate ALS patients. These results suggest that salivary CgA levels may reflect the affective state accurately in these patients. Moreover, we could demonstrate salivary CgA levels in terminal ALS patients in whom the emotional functioning score based on ALSAQ-40 cannot

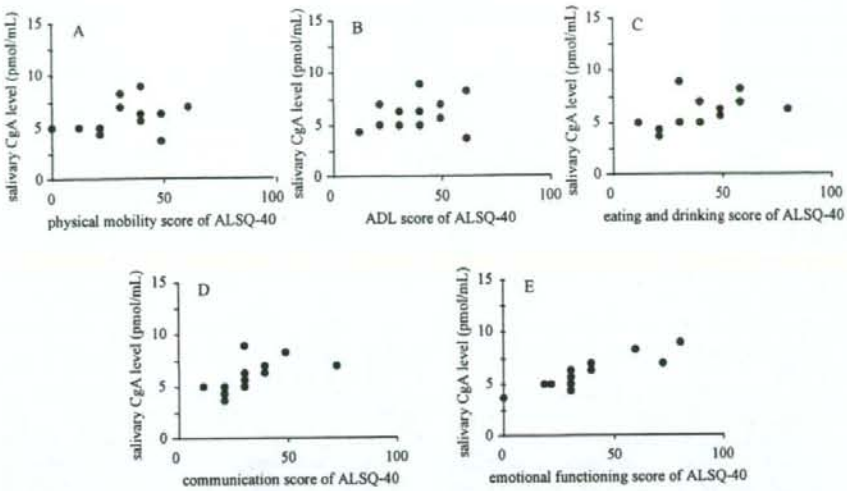

Figure 3. Correlation between salivary CgA levels and physical mobility (A), ADL (B), eating and drinking (C), communication (D), and (E) emotional functioning score of ALSAQ-40 in 12 moderate ALS patients. Although no significant correlation was observed between the salivary CgA level and physical mobility (r=0.412, p>0.1), ADL (r=0.399, p>0.1), eating and drinking (r=0.550, p>0.05), and communication scores (r=0.544, p>0.05) on ALSAQ-40, a statistically significant highly positive correlation was observed between the salivary CgA level and the emotional functioning score in these patients (r=0.892, p<0.01).

be assessed. Thus, salivary CgA may be a useful quantitative biochemical marker of the affective state not only in moderate but also in terminal ALS patients.

There were some individual differences in salivary CgA levels in spite of the similar severity of disease. Two major factors could affect the results: first, autonomic dysfunction in the salivary gland. Salivary secretion of CgA is controlled by the autonomic nervous system (16). Usually, symptoms of disturbed autonomic function are rare in ALS. Indeed, all of our ALS patients did not show the symptoms of autonomic dysfunction, and the data of short-term heart rate variability were also normal in these patients; however, abnormal autonomic tests have been subscribed by a number of researchers in up 40% of patients (17). The mechanism of abnormal autonomic function is not clear, but abnormalities in the axons to dermal vessels and sweat glands, and ultrastructural abnormalities in the sweat glands have been reported (18). Moreover, the neuropathology of some ALS patients with a long-term ventilator shows the involvement of central autonomic network in addition to the upper and lower motor system involvement (19). Abnormalities in the salivary glands and the effect of long-term mechanical ventilation may influence salivary CgA secretion in these patients. The second factor is the influence of personality factors. In this study, some individual differences in dimension scores on ALSAQ-40 were also shown in moderate ALS patients. In addition, Krampe et al reported that being less agreeable might serve as a protective factor with respect to QOL and disease progression in ALS (20). Additional unclarified abnormalities may also influence salivary CgA secretion in these patients.

In this study, the mean salivary CgA level in terminal ALS patients, who were all PEG-tube fed, was significantly higher than in moderate ALS patients and in healthy volunteers, who took meals periorally. However, our correlation studies suggest that the status of food intake did not affect the salivary CgA levels. Mean salivary CgA in patients with vascular dementia, who were also all PEG-tube fed, did not significant differ from those in healthy controls (Fig. 2). Some other stress caused by the progression of the disease may affect the increment of salivary CgA levels in terminal ALS patients.

Although drugs to treat psychological symptoms in ALS patients, such as minor tranquilizers, have been used, the effects on the QOL of these patients are limited. Additional therapies are needed to decrease their stress levels. Recently, Hasson et al reported that salivary CgA decreased significantly in a web-based stress management training group as compared to the control group of healthy volunteers (21). Toda et al reported that the high-stress group of healthy controls showed lower salivary CgA levels after spa bathing (22). Moreover, Suzuki et al reported that salivary CgA levels were significantly decreased after music therapy twice a week for 8 consecutive weeks in elderly patients with dementia (23). Thus, periodic salivary CgA measurements in the long term and/or in various situations may be useful to offer more effective tailor-made psychophysiological therapies for individual ALS patients.