**Rituximab-associated Progressive Multifocal Leukoencephalopathy Derived from Non-Hodgkin Lymphoma: Neuropathological Findings and Results of Mefloquine Treatment**

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**Abstract**

A 66-year-old man with non-Hodgkin lymphoma (NHL) developed progressive multifocal leukoencephalopathy (PML) after undergoing chemotherapy including rituximab. Although the administration of mefloquine at a dose of 500 mg weekly temporarily led to a dramatic decrease in the copy number of JC Virus DNA in the cerebrospinal fluid, the patient’s symptoms gradually worsened. The CD4⁺ T count remained continuously low, at least until approximately five months after the last cycle of chemotherapy. A postmortem examination performed 10 months after the onset of PML disclosed a severe condition associated with rituximab-treated PML originating from NHL and a high mefloquine concentration in the brain. The accumulation of further data regarding mefloquine treatment in PML cases may help to elucidate the optimal dosage and time window for effectively treating PML.

**Key words:** non-Hodgkin lymphoma, progressive multifocal leukoencephalopathy, rituximab, mefloquine, JC polyoma virus

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**Introduction**

Progressive multifocal leukoencephalopathy (PML) is a rare demyelinating disease of the central nervous system that results from the reactivation of latent JC polyoma virus (JCV) (1). PML typically occurs in persons with suppressed cellular immunity, particularly those with HIV infection. In recent years, however, attention has focused on the potential association between PML and monoclonal antibody therapy (2). For example, Carson et al. reported the details of 57 rituximab-associated PML cases involving HIV-negative patients (3) in which 90% of the patients died, with a median time to death after the diagnosis of PML of 2.0 months, thus indicating a poor prognosis of PML associated with rituximab treatment. Recently, the anti-malarial drug mefloquine was found to have an anti-JCV activity in an in vitro assay (4) and subsequently shown to produce some clinical improvements (5-9). However, many factors remain unresolved regarding this drug, including its true efficacy and the optimum dose for treating PML. In addition, the detailed neuropathological findings of rituximab-associated PML patients with non-Hodgkin lymphoma (NHL) have not been reported to date. We herein present the clinical and neuropathological features of rituximab-associated PML in a patient with NHL. In addition, we provide the first informa-
Figure 1. Serial FLAIR magnetic resonance images (MRI) of the brain at approximately two months (A), 3.5 months (B), seven months (C) and nine months (D) after the onset of PML. The first FLAIR images (A) showed hyperintense lesions in the left temporal, parietal and occipital white matter, as well as in the right frontal and occipital lobes (A). These MRI findings (A-D) indicate the serial progression of PML.

A 66-year-old man was diagnosed with the recurrence of CD20-positive diffuse large B-cell lymphoma after two years of remission and underwent four cycles of chemotherapy with cyclophosphamide, vincristine, doxorubicin, prednisolone and rituximab (R-CHOP). At the end of the third cycle of the R-CHOP regimen, he developed right-sided deficits in vision. The patient’s neurological symptoms continued to worsen despite the remission of NHL, and he was subsequently referred to our hospital approximately two months after the onset of the visual disturbance. A neurological examination revealed word amnesia, agraphia, right-sided homonymous hemianopsia and bilateral ideomotor apraxia. Brain magnetic resonance imaging (MRI) showed left parieto-occipital and right frontal hyperintense white matter lesions on fluid-attenuated inversion-recovery (FLAIR) images without gadolinium enhancement (Fig. 1A). CSF exhibited a slightly elevated protein level (54 mg/dL), with a normal cell count (2/μL). Real-time polymerase chain reaction for the JCV in the CSF yielded a positive result of $1.36 \times 10^6$ copies/mL. Other laboratory studies demonstrated negative serological results for HIV infection, with decreased absolute CD4+ T cell (305/μL) and CD19+ cell (3/μL) counts and a normal CD8+ T cell count (596/μL). The serum IgG concentration was 962 mg/dL (reference range: 870-1,700 mg/dL) and remained within the normal range thereafter. Based on the patient’s clinical and MRI findings, as well as the presence of JCV in the CSF, a diagnosis of PML was made according to the PML diagnostic criteria (10). Treatment with 15 mg of mirtazapine per day, which has been reported to be effective in some cases of PML (11, 12), was initiated. In addition, approximately three months after the onset of PML, mefloquine therapy was started at a dose of 500 mg twice weekly then subsequently prescribed at a dose of 250 mg weekly. The treatment with mefloquine was approved by the institutional Ethics Committee at Yamaguchi University Hospital, and we obtained written informed consent from the patient’s family before beginning the therapy. No symptoms or laboratory abnormalities derived from side effects of this drug were noted; however, the patient gradually developed left-sided homonymous hemianopsia and right-sided hemiparesis. Furthermore, brain MRI performed three weeks after the initiation of mefloquine demonstrated expansion of the lesions (Fig. 1B). Four weeks after the initiation of mefloquine, the JCV DNA copy number in the CSF was $1.21 \times 10^6$ copies/
mL, indicating that the therapy against PML was insufficient. The serum and CSF concentrations of mefloquine were examined using high-performance liquid chromatography (HPLC), as previously described (13), approximately four weeks after the initiation of mefloquine, with levels of 0.93 μM and 0.079 μM, respectively. At that time, namely, approximately four months after the onset of PML, a hematological analysis revealed a persistent decrease in both the absolute CD4+ T cell (270/μL) and CD19+ cell (16/μL) counts. Because clinical and radiological progression was observed in spite of the above treatments and the current dose of mefloquine was considered to be insufficient, we increased the dose from 250 mg to 500 mg weekly. As expected, the concentrations of mefloquine in the serum and CSF measured approximately four weeks after the dose increase of mefloquine rose to 2.05 μM and 0.116 μM, respectively, while the JCV DNA copy number decreased to 2.0×10^5 copies/mL. Despite the decrease in the JCV titer in the CSF, the patient’s right-sided hemiparesis progressed and he gradually developed a disturbance of consciousness. Approximately 5.5 months after the onset of PML, persistently low absolute counts of CD4+ (356/μL) and CD19+ (10/μL) cells were observed. Three months after the augmentation of the mefloquine dose and approximately six months after the onset of PML, the JCV DNA copy number finally decreased to 254 copies/mL. In spite of the profound decrease in the JCV load in the CSF, brain MRI performed approximately three months after the increase in the mefloquine dose demonstrated lesion expansion (Fig. 1C), and the patient presented with akinetic mutism. The administration of mefloquine at a dose of 500 mg weekly was continued thereafter, and the JCV DNA copy number increased to 16,800 copies/mL seven months after the augmentation of mefloquine. Approximately eight months after the initiation of mefloquine, the patient died of pneumonia, and an autopsy was subsequently performed. The total clinical course of the PML was approximately 10 months.

Pathological findings

The patient’s brain weighed 1,170 grams. The autopsy showed moderate atrophy in the frontal, parietal and temporal lobes. Large diffuse areas of softening and granular le-
sions were noted in the cerebral white matter on horizontal sections of the cerebrum, with severe loss of white matter and cystic formation in the left posterior lobe. Histopathologically, there were large diffuse areas exhibiting the complete loss of myelin on Hematoxylin and Eosin (HE)-Luxol fast blue (LFB) staining of the cerebral white matter (Fig. 2A). These lesions were not clearly demarcated from the surrounding white matter and displayed negativity for antibodies against myelin basic protein (MBP). Within the center of the large lesions, myelinated fibers were almost absent, accompanied by the severe loss of axons. There were also numerous macrophages containing myelin debris, as well as bizarre astrocytes (Fig. 2B, C). However, no significant infiltration of CD3 or CD8 immunoreactive cells was observed. Although there are currently no definitive neuropathologic criteria for the disease, immune reconstitution inflammatory syndrome (IRIS) is generally characterized by the presence of numerous areas of infiltration of CD8 cells and a low number of VP-1 cells (14, 15). Therefore, we consider that the present case did not show strong evidence of IRIS at the time of autopsy. Meanwhile, there were a few small demyelinated lesions in the brainstem and cerebellum, with spherical or slightly oval densely eosinophilic oligodendroglial nuclei in the periphery of the demyelinated lesions (Fig. 2C). In some instances, the oligodendroglial nuclei exhibited a diffuse basophilic appearance with chromatin particles. These findings were consistent with pathologic changes of PML. Furthermore, the oligodendroglial nuclei showed immunoreactivity for antibodies raised against the VP1 protein (Fig. 2D). However, VP1 immunoreactive deposits were only observed in confined small areas within the peripheral lesions involving a loss of white matter. VP-1-immunoreactive deposits were also present in the cytoplasm of the oligodendroglial cells as well as in the neuropil. Electron micrograms obtained using formalin-fixed wet tissue disclosed intranuclear aggregates of spherical particles in the oligodendroglial cells. However, no lymphoma cells were detected in the central nervous system, peripheral organs or lymph nodes.

**Pharmacological analysis**

We analyzed the concentrations of mefloquine in the cerebrum using the HPLC method. Parts of the cerebral cortex and white matter in the right frontal lobe were obtained at the time of autopsy; the tissue was subsequently frozen and stored until use in the pharmacological analysis. The weight of the cerebral cortex and white matter used in the examination was 0.594 and 0.503 grams, respectively, and the mefloquine concentration in each area was 16.89 and 16.81 μM, respectively.

**Discussion**

To the best of our knowledge, this is the first report to demonstrate the detailed neuropathological findings of rituximab-associated PML in a patient with NHL. The characteristics of the patient’s neuropathological condition included diffuse large demyelinating lesions in the cerebral white matter. This pattern of PML is reminiscent of the neuropathological changes noted in patients with AIDS who did not receive any specific treatments for the HIV virus. In addition, in the present case, there were small demyelinated lesions in both the brainstem and cerebellum. Taking into consideration the patient’s neuropathological findings, extremely high JCV copy number in the CSF during the early clinical course and severe lesions on repeated brain MRI examinations (Fig. 1), we believe that rituximab-associated PML, particularly that originating from NHL, is a very severe disease that may be difficult to treat. In the current case, VP-1-immunoreactive glial cells were observed in relatively limited regions of the cerebrum. However, it was difficult to conclude whether the viral activity was well controlled during the patient’s clinical course based only on the neuropathological findings. Nevertheless, the decrease in the JCV copy number in the CSF and the relative confinement of the VP-1-immunoreactive cells suggest that mefloquine was somewhat effective with regard to suppressing the JCV infection in the central nervous system.

In the present case, the absolute count of CD4+ T cells remained continuously low (276-305/μL), at least until approximately five months after the last cycle of chemotherapy including rituximab. Despite the persistently low level of CD4+ cells, the copy number of JCV decreased from 1.21×10⁶ copies/mL to 254 copies/mL after the dose of mefloquine was increased. Although the treatment with mirtazapine may have played a slight role in suppressing the JCV in this case, we believe that the obvious decline in the copy number of JCV in the CSF was brought about primarily by the mefloquine therapy because the decrease in the JCV titer began only after the dose of this drug was increased, even under the patient’s state of defective cellular immunity. On the other hand, it is unclear why PML developed in spite of the decline in the copy number of JCV in the CSF. It is possible that the JC virus survived in small numbers and continued to destroy the CNS under conditions of immunosuppression. Laszlo et al. reported that, in their study, the CD4+ cell count decreased to a median of 216 CD4+ cells among NHL patients who received rituximab and chlorambucil and failed to recover to within the normal range in 50% of the patients at a median follow-up of nine months (3-30 months) from the end of treatment (16). In addition, Carson et al. reported that seven of nine NHL patients treated with chemotherapy including rituximab had a CD4+ cell count less than 500/μL (3); among these seven patients, five had a CD4+ cell count less than 200/μL. However, that report did not include long-term follow-up of the CD4+ cell count, and how long a low level of CD4+ cells persists in NHL patients treated with chemotherapy containing rituximab has not yet been elucidated. More information regarding the CD4+ cell count in PML patients who receive chemotherapy with rituximab is strongly needed.

The serum concentration of mefloquine in our patient,
who received this drug at a dose of 250 mg weekly, remained below (0.93 μM) that (1-5 μM) observed in healthy subjects treated with a dose of 250 mg weekly (17). After increasing the dose of mefloquine from 250 mg to 500 mg, the serum concentration barely reached 2.05 μM. Therefore, the measuring the serum concentration of mefloquine is very important, as the range of the concentration of this drug in PML patients may be relatively wide. In one study, the concentrations of mefloquine in the brain of four individuals who received the drug at a dose of 250 mg weekly were approximately 23-37 μM (18). In addition, Brickelmaier et al. demonstrated that mefloquine reduces the number of viral copies in infected human primary cultured glial cells by 50% or more at a concentration of 4 μM (4). Hence, the approved doses of mefloquine are presumed to be sufficient for treating PML. In the current case, the concentration in the brain was 16.8 μM, which was much higher than the effective concentration of 4 μM estimated based on an in vitro study (4). Considering that mefloquine displays a relatively good transfer rate across the blood-brain barrier, it is reasonable that the concentration of this drug in our patient’s brain was greater than 4 μM under treatment at a dose of 250 mg weekly. However, the JCV copy number declined only after the dose of the drug was increased in this case. This finding indicates the possibility that the truly effective concentration of mefloquine in the PML brain is much higher than 4 μM. Taken together, the weekly administration of 250 mg of mefloquine as a maintenance dose may be insufficient for suppressing the activity of JC viruses in the brain in cases of PML, particularly rituximab-associated cases derived from NHL, and a weekly dose of 500 mg or higher may therefore be suitable for treating PML in patients with a severe condition.

Although several cases of PML, in which mefloquine was shown to be effective have been reported (5-9), recent reports have indicated a failure to reduce the JCV DNA titer in the CSF (19-21). At present, the differences in the backgrounds of PML patients between responders and non-responders to mefloquine have not been elucidated. Furthermore, it is unclear whether the effectiveness of this drug in cases of PML is concentration-dependent. Since the reported median time to death after the diagnosis of PML in patients with rituximab-associated PML is 2.0 months (3), the survival period of 10 months after the onset of PML observed in the current case suggests that mefloquine was effective with regard to extending the survival of our patient. The accumulation of PML cases investigating the distribution of mefloquine, as well as further elucidation of the correlation between the concentrations of the drug in the brain and CSF, may lead to a better understanding of the efficacy of this drug as PML therapy.

The authors state that they have no Conflict of Interest (COI).

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