Progressive Retinitis-Encephalitis Due to Ganciclovir-Resistant Cytomegalovirus Associated with Aplastic Anemia

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A 29-year-old woman with aplastic anemia who complained of visual disturbances and pain in the right eyeball was diagnosed as having cytomegalovirus (CMV) retinitis based upon the characteristic retinal changes and isolation of CMV. She received treatment with ganciclovir (GCV), and the retinitis initially responded for several months. However, the patient was found to have CMV lesions in the left eye followed by neurological symptoms. The CMV isolated just before her death was approximately 20 times more resistant to GCV than that isolated previously, suggesting that the GCV-resistant CMV had developed during the long-term treatment with GCV. (Internal Medicine 36: 375-379, 1997)

Key words: non-acquired immunodeficiency syndrome (non-AIDS) patient, autopsy, polymerase chain reaction (PCR)

Introduction

After primary infection, cytomegalovirus (CMV) remains latent within the host, who is usually asymptomatic. Under immunosuppression, however, the latent virus may be reactivated, producing a variety of clinical manifestations, including retinitis, pneumonia, and encephalitis (1, 2). The clinical manifestations are especially common in cases with cell-mediated immunity impairment such as organ recipients and patients with acquired immunodeficiency syndrome (AIDS) (3). Ganciclovir (GCV) is currently the only drug available for the treatment of CMV infection in Japan (4). However, it was reported that the GCV-resistant virus emerged in AIDS patients who had received this agent for extended periods (5). In this report, we present a case of severe CMV infection associated with aplastic anemia, in which a GCV-resistant strain was isolated.

Case Report

The patient was a 29-year-old who consulted us because of visual disturbances and other symptoms in 1991. In 1984, when she was 22 years old and pregnant, she was found to have severe anemia and moderate thrombocytopenia. The diagnosis of

aplastic anemia was made based upon the finding of hypoplastic bone marrow. She was treated with 5–10 mg of prednisolone daily. Although she had moderate anemia (hemoglobin concentrations, 7–10 g/dl) and moderate thrombocytopenia (platelet count, $50\text{--}70 \times 10^9/l$), she did not experience any major limitation of her daily activities.

She was admitted to our hospital in April 1991, with the complaints of visual disturbances, pain in the right eyeball, and a high fever. She was found to have moderate anemia (hemoglobin concentration, 7.4 g/dl), thrombocytopenia (platelet count, $54 \times 10^9/l$), and total leukocyte count of $3.4 \times 10^9/l$ (31.3% neutrophils and 55.1% lymphocytes). The bone marrow examination revealed hypocellularity without any abnormal cells. She tested positive for human T-cell lymphotropic virus type-1 (HTLV-1) antibody (titer, 1:2,560). Human immunodeficiency virus (HIV) antibody was negative, although her peripheral blood CD4/8 ratio was decreased to 0.5. The level of C-reactive protein in peripheral blood was increased to 3.6 mg/dl. Both liver and kidney functions tested normal. Ophthalmologic examination revealed a creamy white granular area with perivascular exudate and hemorrhage to the right eye (Fig. 1). CMV was isolated from the peripheral blood, urine, and tears. The diagnosis of CMV retinitis was made based on the characteristic retinal change and isolation of CMV.

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Figure 1. Ophthalmologic appearance of the fundus of the right eye. Creamy white granular lesions with perivascular exudate and hemorrhage are observed.

High-titer anti-CMV globulin was administered, in consideration of the patient's aplastic anemia, until August 1991. As the retinal lesions progressed gradually, however, treatment with GCV at a reduced dosage (1.5–5.0 mg/kg body weight), to avoid severe pancytopenia, was begun (Fig. 2). The retinitis responded to this treatment, and the culture test for CMV became negative within a few months. However, because pancytopenia developed during GCV treatment, the dosage of GCV was reduced and granulocyte-colony stimulating factor was used temporarily. The retinitis then recurred and progressed with sporadic fever and general malaise, although the patient's general condition was fairly good.

In July 1992, the patient recognized visual disturbances in the left eye. Because retinal lesions of the left eye similar to those of the right eye were observed, the dosage of GCV was increased to 5 mg/kg daily. However, the lesions of both fundi did not improve, and the blood culture tests for CMV became consistently positive. In October, the patient complained of dizziness and hearing disturbances in the right ear, followed in December by occurrence of speech disturbance and consciousness disturbance. T₂-weighted magnetic resonance images (MRI) of the head revealed the presence of multiple high-intensity spot lesions in the basal ganglia, pons and white matter deep to the

Figure 2. The patient's clinical course. CMV: cytomegalovirus, Plt: platelet, WBC: white blood cell.

cerebral cortex (Fig. 3). According to these findings, the patient was diagnosed as having CMV encephalitis. In spite of the administration at high dosage of GCV, the patient did not recover and died in January 1993.

Pathological findings

Pathological examination at autopsy revealed the presence of giant degenerative cells with intranuclear inclusion bodies throughout the entire brain. These giant cells were particularly abundant in the optic nerve, optic chiasm and optic tract. Immunological studies using an anti-CMV monoclonal antibody (HRP-C7) demonstrated that cells positive for CMV antigen were present even in the areas where giant cells were absent (Fig. 4A and 4B) (6). Examination of the lung revealed pneumonia due to coinfection of CMV with *Pneumocystis carinii*, as well as pulmonary bleeding.

Virological findings

DNA was extracted from the various organs obtained at autopsy. CMV DNA was examined by the polymerase chain reaction (PCR) method using the primers for the fourth exon of the coding region for major immediate early antigen (7). As shown in Fig. 5, the 426 bp CMV-specific band was positive in the cerebrum, mesencephalon, pons, lung, liver, kidney, and spleen. The susceptibilities of CMV samples isolated from the peripheral blood at two different times were compared using a 50% plaque-reduction test (Fig. 6) (8). The CMV isolated just

Figure 3. T₂-weighted magnetic resonance image of the head. Multiple high-intensity spot lesions are observed in the basal ganglia and the white matter deep to the cerebral cortex (arrows).

Figure 4. CMV-infected cells (A) (HE stain, $\times 200$) and CMV antigen-positive cells (B) (immunohistochemical stain, $\times 200$) in the brain. CMV-infected cells were found scattered throughout the whole brain and CMV antigen was localized in both CMV-infected giant cells and glial cells.

Figure 5. Amplification of CMV DNA sequences. One microgram of DNA extracted from each of the various organs indicated was used as a template. The CMV-specific DNA band was observed to be the same size as that of the positive control, AD169, using the primers for the 4th exon of the major immediate-early gene. The size marker is shown in the far left lane. Bp, base pairs.

Figure 6. Susceptibility test results for CMV to ganciclovir. CMV isolates obtained at the two times indicated were used. The inhibitory concentration (ED_{50}) was defined as the concentration that resulted in a 50% decrease in plaque number compared with the control (8).

before death was approximately twenty times more resistant than that isolated before the GCV treatment. The CMV isolated at both times showed the same restriction endonuclease cleavage pattern when digested with *EcoR* I, *Hind* III or *Pst* I (data not shown). Therefore, it is likely that the GCV-resistant CMV strain developed from a GCV-sensitive strain in the patient during the long-term administration of GCV.

Discussion

The retinitis and encephalitis due to CMV generally occur in severely immunocompromised adult patients, such as those with AIDS and receiving immunosuppressive drugs. Although the present patient had aplastic anemia prior to the onset of CMV retinitis, the severity of the disease was mild. She was negative for HIV. She was not suffering from any severe infection except CMV until the terminal stage of the disease. She had received prednisolone treatment for her aplastic anemia for several years, although the amount of prednisolone given had been small. Therefore, it is possible that the long-term treatment with prednisolone caused an immunosuppressive state resembling AIDS, because her CD4/CD8 was already low even at the onset of retinitis. Alternatively, the infection with HTLV-1 in this patient might have affected her cellular immunity, as HTLV-1 infection is reported to cause the anergy of tuberculin test (9).

Encephalitis associated with CMV retinitis frequently progresses. Bylsma SS et al (10) reported that 91% of AIDS patients with CMV encephalitis have concurrent retinitis and 10% of the patients with CMV retinitis develop encephalitis. Both diffuse micronodular encephalitis and ventriculoencephalitis are reported to occur frequently in patients receiving maintenance therapy for CMV retinitis, and as the mechanism for this phenomenon inadequate penetration of GCV into the brain parenchyma or selection of CMV resistant to GCV has been suggested (11).

The present patient was treated for 18 months with GCV, which is currently the only authorized anti-CMV reagent in Japan. It is well recognized that CMV resistance to GCV

commonly develops with prolonged use. Drew WL et al (5) reported that 38% of CMV samples isolated from CMV-infected patients were resistant to GCV after treatment with GCV for more than 3 months. We suspect that a GCV-resistant virus developed in the present patient, in whom the bilateral CMV retinitis required the continued use of GCV. Moreover, the CMV isolated from our patient at the terminal stage was approximately 20 times more resistant (28 μ M for 50% plaque-reduction) to GCV than that isolated before the CMV treatment (1.3 μ M). As the CMV isolates resistant to GCV are defined as those requiring $>5 \mu$ M GCV for the inhibition of 50% of viral plaques (12), the CMV isolated from the patient at terminal stage can be regarded as very resistant to GCV. The presence of GCV-resistant virus in some patients before treatment with GCV has been reported (8). In our patient, the CMV obtained at two times both showed the same restriction endonuclease cleavage pattern, indicating that both were of the same strain. The dosage of GCV that could be tolerated in this patient was limited, given her aplastic aemia. The limitation of dosage for the long-term treatment might have accelerated the selection of GCV-resistant mutant CMV.

Because of the increasing numbers of cases of AIDS and organ transplantation, it is likely that severe CMV infection will become more frequent, even in Japanese adults. CMV infection in immunocompromised hosts is usually treated with GCV for long periods, resulting in the frequent emergence of GCV-resistant CMV. Another anti-CMV reagent, foscarnet, which is currently unavailable in Japan, has been reported to be effective against GCV-resistant CMV (13, 14). Thus, a combination therapy of GCV with foscarnet should be considered for severe CMV infection.

References

- 1) Monto H. Cytomegalovirus. in Principles and practice of infectious

- disease (Third Edition), Mandell GL, Douglas RG, Bennett JE, Eds. Churchill Livingstone Inc, New York, 1990, p 1159.
- 2) Zaia JA. Epidemiology and pathogenesis of cytomegalovirus disease. *Semin Hematol* **27**: 5, 1990.
- 3) Britt WJ, Alford CA. Cytomegalovirus. in Fields Virology, Third Edition, Fields BN, Knipe DM, Howley PM, et al, Eds. Lippincott-Raven Publishers, Philadelphia, 1996, p. 2506
- 4) Matthews T, Boehme R. Antiviral activity and mechanism of action of ganciclovir. *Rev Infect Dis* **10** Suppl 3: S490, 1988
- 5) Drew WL, Miner RC, Busch DF, et al. Prevalence of resistance in patients receiving ganciclovir for serious cytomegalovirus infection. *J Infect Dis* **163**: 716, 1991
- 6) Eizuru Y, Minematsu T, Minamishima Y, et al. Rapid diagnosis of cytomegalovirus infections by direct immunoperoxidase staining with human monoclonal antibody against an immediate-early antigen. *Microbiol Immunol* **35**: 1015, 1991
- 7) Yamaguchi Y, Hironaka T, Kajiwara M, Tateno E, Kita H, Hirai K. Increased sensitivity for detection of human cytomegalovirus in urine by removal of inhibitors for the polymerase chain reaction. *J Virol Methods* **37**: 209, 1992
- 8) Erice A, Chou S, Biron KK, Stanat SC, Balfour HH Jr, Jordan MC. Progressive disease due to ganciclovir-resistant cytomegalovirus in immunocompromised patients. *N Engl J Med* **320**: 289, 1989
- 9) Murai K, Tachibana N, Shioiri S, et al. Suppression of delayed-type hypersensitivity to PPD and PHA in elderly HTLV-I carriers. *J Acquir Immune Defic Syndr* **3**: 1006, 1990.
- 10) Bylsma SS, Achim CL, Wiley CA, et al. The predictive value of cytomegalovirus retinitis for cytomegalovirus encephalitis in acquired immunodeficiency syndrome. *Arch Ophthalmol* **113**: 89, 1995.
- 11) McCutchan JA. Cytomegalovirus infections of the nervous system in patients with AIDS. *Clin Infect Dis* **20**: 747, 1995.
- 12) Drew WL. Resistance of cytomegalovirus to antivirals. *Int Antiviral News* **2**: 114, 1994.
- 13) Studies of Ocular Complications of AIDS Research Group in collaboration with the AIDS Clinical Trials Group. Mortality in patients with the acquired immunodeficiency syndrome treated with either foscarnet or ganciclovir for cytomegalovirus retinitis. *N Engl J Med* **326**: 213, 1992 (published erratum appears in *N Engl J Med* **326**: 1172, 1992) (see comments).
- 14) Drew WL. Nonpulmonary manifestations of cytomegalovirus infection in immunocompromised patients. *Clin Microbiol Rev* **5**: 204, 1992.