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Successful Treatment of Neonatal Herpes Simplex-Type 1 Infection Complicated by Hemophagocytic Lymphohistiocytosis and Acute Liver Failure

Keitaro Yamada,[1,2] Yutaka Yamamoto,[1,2] Atsushi Uchiyama,[1] Reiko Ito,[3] Yusuke Aoki,[2,4] Yasushi Uchida,[2,4] Hiroyuki Nagasawa,[1] Hiroshi Kimura,[5] Takashi Ichiyama,[6] Toshiyuki Fukao[2] and Yoshinori Kohno[1]

[1]Department of Neonatology, Gifu Prefectural General Medical Center, Gifu, Japan
[2]Department of Pediatrics, Graduate School of Medicine, Gifu University, Gifu, Japan
[3]Department of Pediatrics, Gifu Prefectural General Medical Center, Gifu, Japan
[4]Department of Pediatrics, Nagara Medical Center, Gifu, Japan
[5]Department of Microbiology, Graduate School of Medicine, Nagoya University, Nagoya, Japan
[6]Department of Pediatrics, Graduate School of Medicine, Yamaguchi University, Yamaguchi, Japan

Neonatal disseminated herpes simplex virus (HSV) infection with acute liver failure (ALF) and neonatal hemophagocytic lymphohistiocytosis (HLH) are severe diseases. We recently experienced a male infant with HLH and ALF induced by HSV type 1 (HSV-1). The infant, born at 39 weeks of gestation by normal delivery, developed a fever on day 4. On day 9, laboratory investigations showed progressive liver dysfunction and coagulopathy, and the serum ferritin was excessively elevated. Furthermore, the blood levels of interleukin (IL)-6, IL-10, and interferon-gamma were also elevated. HSV-1 DNA was detected in the serum and cerebrospinal fluid by the real-time PCR method. A diagnosis of HLH was established based upon the following criteria: fever, splenomegaly, cytopenia (two cell lines), serum ferritin (> 500 μg/l) and hypofibrinogenemia (< 150 mg/dl). High-dose acyclovir therapy, steroid pulse therapy using methylprednisolone, high-dose gamma globulin therapy and a blood transfusion were given. The patient recovered without neurological deficit. Neonatal disseminated HSV infections may be complicated by the development of HLH and hypercyokinemia. If HLH is suspected, not only high-dose acyclovir therapy but also anti-cytokine therapy should be considered. ———— newborn; herpes simplex virus type 1 infection; hemophagocytic lymphohistiocytosis; hypercytokinemia; acute liver failure.
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Neonatal herpes simplex virus (HSV) infection is a severe disease with a high incidence of mortality and morbidity (Kimberlin 2004). It may be associated with hyper inflammatory cytokinemia (Kawada et al. 2004). Hemophagocytic lymphohistiocytosis (HLH) is characterized by fever, hepatosplenomegaly, central nervous system symptoms, cytopenias, coagulopathy and lipid changes which may be the result of hypercytokinemia, and tissue and organ infiltration by

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Correspondence: Keitaro Yamada, Department of Pediatrics, Graduate School of Medicine, Gifu University, 1-1 Yanagido, Gifu 501-1194, Japan.
e-mail: keitaro_ymd1976@yahoo.co.jp

hemophagocytic histiocytes (Janka et al. 2004). We recently had a patient with neonatal HLH and acute liver failure (ALF) induced by disseminated HSV type 1 (HSV-1) infection. The patient was successfully treated with high-dose acyclovir and anti-cytokine therapy.

Clinical Findings

A male infant was born at 39 weeks of gestation by normal delivery weighing 3.0 kg and was subsequently breast-fed. There was no history of maternal primary herpetic genital lesions, the parents were healthy and non consanguineous, and no family history of HLH was noted.

The infant developed fever (38.5°C) on day 4 after birth and was transferred to a local hospital on day 5. Initial investigations showed a white blood cell count of 10,000/μl (71% neutrophils), hemoglobin 14.6 g/dl, platelet 20.7 × $10^4/\mu$l, C-reactive protein 2.3 mg/dl, aspartate aminotransferase (AST) 611 IU/l, alanine aminotransferase (ALT) 156 IU/l and lactic dehydrogenase (LDH) 1,839 IU/l. A cerebrospinal fluid (CSF) test showed that the white blood cell count and the protein concentration were not elevated. He was tentatively diagnosed as having a bacterial infection with liver dysfunction, and was given intravenous antibiotics. The hepatitis A, B, and C viruses, Epstein-Barr virus, cytomegalovirus, rubella infections and toxoplasmosis were excluded using virological or serological methods. On day 9 after birth, the real-time PCR method for HSV-1 DNA revealed 3.2 × 10^6 copies/ml serum and 4.5 × 10^3 copies/ml CSF. Progressive liver dysfunction developed; the laboratory findings were a total bilirubin (T.bil) of 2.8 mg/dl, direct bilirubin (D.bil) of 0.7 mg/dl, AST 3,237 IU/l, ALT 851 IU/l, LDH 7,153 IU/l, albumin 2.8 g/dl and ammonia 97 μg/dl. In addition, coagulopathy was manifested by a prolonged prothrombin time

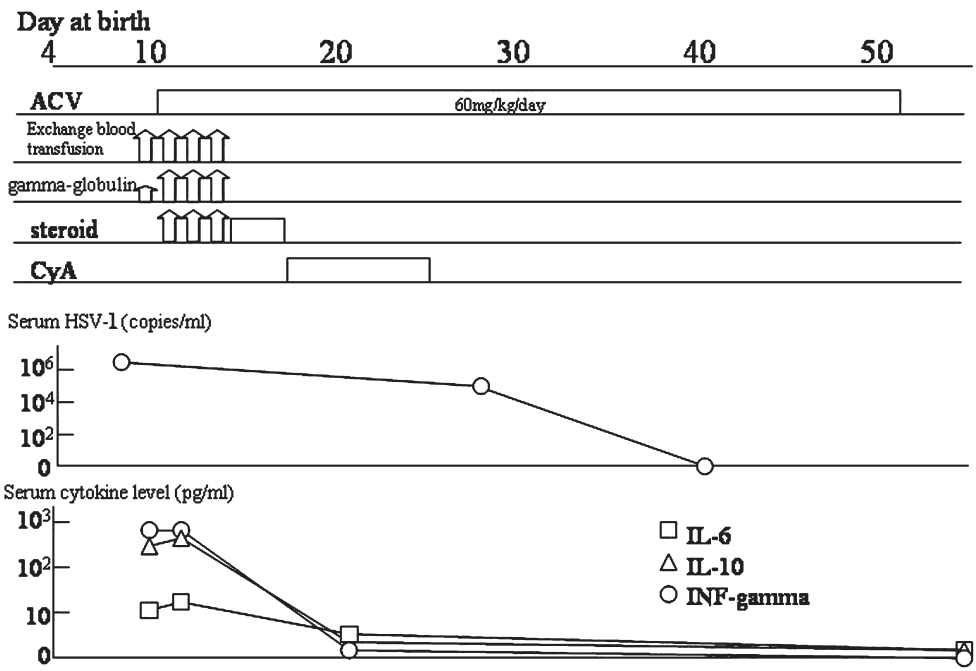

Fig. 1. Clinical course.
Main treatments and changes of serum HSV-1 DNA and cytokine levels are shown. Treatments with high-dose acyclovir (60 mg/kg/day), steroid pulse therapy (methylpredonisolone 30 mg/kg/day), high-dose gamma globulin therapy (1 g/kg/day) and exchange blood transfusion were started from day 9 after birth. Serum HSV-1 DNA and serum cytokine levels of IL-6, IL-10 and IFN-gamma decreased as the clinical course got better.
ACV, acyclovir; CyA, cyclosporine.

of 26.5 sec (reference time 11.2 sec), activated partial thromboplastin time of 66 sec (reference time 31.3 sec), hepaplastin test 42%, fibrinogen 77 mg/dl and thrombocytopenia (platelet 3.9 × $10^4/\mu$l). In addition, serum ferritin (> 15,000 μg/l) and a soluble interleukin (IL)-2 receptor (2,216 IU/l) were abnormally elevated. Based on these findings, the diagnosis of disseminated HSV-1 infection with hepatic failure was made and treatment with high-dose acyclovir (60 mg/kg/day) was started. In addition, steroid pulse therapy with methylprednisolone (30 mg/kg/day), high-dose gamma globulin (1 g/kg/day) therapy and exchange blood transfusion were given for 3 days from day 9 after birth. Subsequently, the infant was transferred to our hospital 14 days after birth since he did not improve with this intensive therapy (Fig. 1).

On admission, he was edematous and unconscious (Glasgow Coma Scale, 3-3-2) with opisthotonic posture and general hypertonia. The abdomen was distended, and the liver and spleen were palpable at 4 cm and 3 cm, respectively, below the costal margin. Umbilical cord bleeding was observed. Anemia (Hb 7.7 g/dl), thrombocytopenia (platelets 4.5 × $10^4/\mu$l), coagulopathy [prothrombin time 124.0 sec (reference time 11.2 sec), activated partial thromboplastin time 74.0 sec (reference time 32.6 sec), hepaplastin test 16%, fibrinogen < 50 mg/dl], hyper-ammoniemia (228 μg/dl), low albumin (2.6 mg/dl), and liver dysfunction with obstructive jaundice (T.bil 5.7 mg/dl, D.bil 3.8 mg/dl, AST 2055 IU/l, ALT 943 IU/l, LDH 4102 IU/l, cholinesterase 236 IU/l, gamma-glutamyltranspeptitase 110 IU/l) were also noted. The diagnosis of HLH was further confirmed since five of eight criteria (HLH2004) required were met: namely, fever, splenomegaly, cytopenia (two cell lines), ferritin (> 500 μg/l) and hypofibrinogenemia (< 150 mg/dl) (Janka and Schneider 2004). Intensive supportive therapy consisting of platelet replacement, fresh frozen plasma, and the administration of acyclovir and antibiotics were continued. Cylosporine A treatment was started on day 16 after birth but discontinued on day 22 since acute renal failure occurred probably due to the drug, which required ventilation and inotropic support (Yamada et al. 2007). Initially, bone marrow analysis could not be performed because of his poor clinical condition and hemophagocytes were not present on day 23 after birth. Acyclovir treatment was discontinued on day 47 after birth since HSV-1 could not be detected in the blood and CSF by the PCR method. He gradually improved neurologically and was discharged on day 83 in spite of still having mild liver dysfunction (T.Bil 10.51 mg/dl, D.Bil 8.41 mg/dl, AST 280 IU/l, ALT 180 IU/l, LDH 407 IU/l). A liver biopsy on day 154 revealed post-necrotic fibrosis, patchy coagulative parenchymal necrosis, striking hepatocellular/canalicular cholestasis, and patchy ballooning of surviving hepatocytes. He is now 1 year old and his growth and development are within normal ranges but the mild liver dysfunction persists (T.bil 0.31 mg/dl, AST 56 IU/l, ALT 62 IU/l, and LDH 257 IU/l).

Serum cytokines at day 8, 10, 17 and 50 after

Table 1. Serum cytokine levels of clinical course.

Day after birth	8	10	17	50
IL-2 (pg/ml, < 4.5)	3.0	< 2.6	10.7	8.5
IL-4 (pg/ml, < 15.0)	< 2.6	< 2.6	3.9	< 2.6
IL-6 (pg/ml, < 19.9)	143.8	194.5	30.3	13.5
IL-10 (pg/ml, < 14.2)	380.3	434.3	15.3	8.6
IFN-gamma (pg/ml, < 42.9)	699.1	693.1	25	9.6
TNF-alpha (pg/ml, < 11.1)	2.9	< 2.8	< 2.8	< 2.8

IL, interleukin; IFN, interferon; TNF, tumor necrosis factor.
Reference values were obtained from Ichiyama et al. (2004).

birth were assayed (Table 1), as described by Ichiyama et al. (2004). The serum levels of IL-6, IL-10 and interferon (IFN)-gamma were elevated at the onset of his illness but decreased as the clinical course improved.

Discussion

We presented here a neonatal patient with disseminated HSV-1 infection which resulted in ALF and HLH. The patient successfully recovered from these serious conditions.

Neonatal HLH is a rare and potentially fatal disorder resulting from abnormal proliferation of histiocytes. Isaacs (2006) reported 72 fetuses and neonates with HLH, among whom about one half were classified as familial HLH, and 17 (24%) as HLH associated with infections, such as cytomegalovirus, rubella, Ebstein-Barr, and enterovirus. However, no examples with HSV were described.

Hemophagocytes are sometimes detected in cases of severe neonatal HSV infections. Imashuku et al. (2000) reported three cases of severe neonatal HSV infection similar to our case. All of them developed a fever on days 2-3 after birth, followed by severe liver damage on days 7-8, and consequently died of multiple organ failure. Hemophagocytes were found in their peripheral blood smears or bone marrow. Verma et al. (2006) reported 11 neonates affected with HSV-induced ALF. Five of them showed a mild degree of hemophagocytosis upon bone marrow examination and only two survived. Although the diagnostic criteria of HLH are not always fulfilled in patients with severe neonatal HSV infection, the diagnosis of HSV-related HLH and/or ALF should be seriously considered because of the poor prognosis of these conditions and physicians should therefore treat them vigorously.

The poor prognosis of severe neonatal HLH and/or ALF in disseminated HSV infection may be associated with a cytokine storm which is also observed in HLH with the other etiologies. The clinical manifestations of HLH are believed to be caused by a hyper inflammatory syndrome because of hypercytokinemia of various pro-inflammatory mediators such as TNF-alpha, IL-6, IL-8, IL-12, IL-18, IFN-gamma, and a number of hematopoietic growth factors released by stimulated lymphocytes and histiocytes (Janka and Schneider 2004). Kawada et al. (2004) reported that serum concentrations of inflammatory cytokines (IL-6, soluble tumor necrosis factor receptor 1) and markers of apotosis (Cytochrome C) were elevated in patients with disseminated HSV infection. Elevated serum levels of IL-6, IL-10 and IFN-gamma were accordingly detected in our patient at the onset.

The treatment of HLH is aimed at the suppression of the severe hyper inflammation and at the eradication of pathogen-infected antigen-presenting cells. In our patient, anti-cytokine therapy, such as exchange blood transfusions, steroid pulse therapy and high-dose immunoglobulin therapy were used in the treatment of HLH, and high-dose acyclovir therapy was given for disseminated HSV infection. Furthermore, his condition was complicated with ALF which required supportive therapy, such as fresh frozen plasma and lactulose. HLH or disseminated HSV infection complicated with ALF have a high mortality rate despite aggressive supportive treatment (Janka and Schneider 2004). Although the early symptoms of disseminated HSV infection are often non-specific and the majority of the mothers who transmit the disease lack a history of genital herpes (Brown et al. 2003), early initiation of antiviral therapy should be considered in neonates with suspected sepsis, particularly in the presence of abnormal liver dysfunction (Kimberlin 2004).

Our patient was an usual case of a successful course in HLH complicated with ALF induced by HSV infection. Our patient recovered from liver failure without liver transplantation and showed no neurological deficit. Since neonatal disseminated HSV infection may be complicated by the hypercytokinemia of HLH, not only high-dose acyclovir therapy but also anti-cytokine therapy should be considered.

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