Effect of Glycyrrhizin in Children with Liver Dysfunction Associated with Cytomegalovirus Infection

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Glycyrrhizin (GL) has an inhibitory effect on several viruses including human immunodeficiency virus type 1 (HIV-1) and varicella-zoster virus (VZV). In addition, some therapeutic and prophylactic effects on chronic active viral hepatitis have been claimed for GL. In this study, 0.2% GL dissolved in saline (2 mg/ml GL), supplemented with 2% glycine and 0.1% cysteine (Stronger Neo-Minophagen C, SNMC) was administered intravenously in a dose of 50 ml/day for a period of more than one week to three infants with cytomegalovirus (CMV) infection who exhibited abnormal liver function or hepatomegaly. Liver function had become normal at the end of the course of SNMC. These findings suggest that GL might have therapeutic effects on liver dysfunction associated with CMV infections.

Glycyrrhizin (GL) consists of one molecule of glycyrrhetic acid (GA) and two molecules of glucuronic acid, and has an anti-inflammatory effect (Ishikawa et al. 1990). GL has been reported to inhibit a number of viruses in vitro and in vivo (Ohtsuki and Ishida 1988). Since the therapeutic and prophylactic efficacy of GL with respect to chronic viral hepatitis has been reported (Takita and Shin 1989), the antiviral effect of this drug to several viruses has been investigated (Hattori et al. 1989; Ito et al. 1989), and it was recently reported that GL also has antiviral effects to varicella-zoster virus (VZV) (Baba and Shigeta 1987). Cytomegalovirus (CMV) remains the most common cause of congenital and perinatal viral infection, a significant cause of transfusion-acquired infections, and a frequent contributor to morbidity and mortality among organ transplant recipients as well as patients who receive chemotherapy or high-dose corticosteroids or

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who are infected with human immunodeficiency virus type 1 (HIV-1) (Forbes 1989). The vast majority of congenital and perinatal CMV infections are chronic, subclinical forms, but symptomatic infections are sufficiently prevalent and dangerous to present a major unsolved public health problem throughout the world (Alford et al. 1990). CMV has been recognized as one of the etiologic agents of neonatal and infantile hepatitis (Chang et al. 1992). No adequate studies on the use of ganciclovir or other anti-CMV agents in the treatment of congenital or perinatal CMV infections have been conducted. Successful treatment with anti-CMV agents unaccompanied by cytotoxicity has yet to be achieved in the field of pediatrics. In this study, we tried to treat the children with abnormal liver function or hepatomegaly associated with CMV infection by intravenous administration of GL.

MATERIALS AND METHODS

Patients

Three infants admitted to Hokkaido Children's Hospital or Sapporo Medical University Hospital were investigated. CMV was isolated from urine samples from these three infants using tissue cultures. Although IgG and IgM antibodies to CMV were detected with ELISA in sera obtained from all three patients, positive results were also obtained using the commercially-based indirect immunofluorescence test. GL was administered by continuous intravenous drip infusion to each patient, the preparation consisting 0.2% GL dissolved in saline (2 mg/mL of GL), supplemented with 2% glycine and 0.1% cysteine (Stronger Neo-Minophagen C, SNMC, kindly provided by Minophagen Pharmaceutical Co., Tokyo) (Takita and Shin 1989). Administration of 50 ml/day of SNMC was decided from the results of pharmacokinetic study of GL (Tanaka et al. 1993). Informed consent was obtained from the parents of each patient prior to treatment.

Monitoring patient status

Sera obtained upon admission and during treatment were assessed for IgG and IgM antibodies to CMV using ELISA kits (Enzygnost, Behringwerke AG, Marburg, Germany) according to the manufacturer's instructions. IgG antibodies to CMV were determined at a dilution of 1 : 100 and IgM antibodies at 1 : 40. The cut-off value for these assays is an 0.200 optical density (O.D.) (Numazaki et al. 1992). Urine samples were also obtained from the patients. Clinical specimens were inoculated onto monolayers of human embryonic lung (HEL) cells to test for cytopathological effect (CPE). The following laboratory tests were performed: OKT4/OKT8 (flow cytometry), OKT4-positive lymphocyte count (flow cytometry), liver function tests, renal function tests, and serum electrolytes.

RESULTS

Case 1 was a 2-month-old girl with poor body weight gain and respiratory disorders (Fig. 1). There were no previous problems in the neonatal period other than respiratory disorders, and although weight gain had been slow during breast feeding, her general condition was good. The patient's mother had IgG antibodies to CMV. There were no episodes of infection during pregnancy. After normal delivery (40 weeks of gestation and a birth weight of 2,880 g), the patient was slightly dyspneic. When she was 60 days old, the patient was admitted with
a history of severe dyspnea and stridor. On admission her pulse rate was 170/min, respiratory rate 60/min, and oral temperature 37.2°C. Her weight was 4.1 kg, body length 58.9 cm and head circumference 35.0 cm. Physical examination revealed a pale, cyanotic, chronically ill-looking girl with stridor and dyspnea. There was mild inflammation of the pharynx but no exudate. The cardiovascular examination was normal. The abdominal examination showed slight hepatosplenomegaly. No superficial lymph nodes were palpable and a vesicourethral fistula was detected. Laryngomalacia was also detected on laryngoscopy. No evidence of inflammation was observed at the time of admission. Computed tomography revealed subdural effusion. The patient was subsequently intubated. When examined on hospital day 7, the following enzymes were elevated: serum glutamate-oxalate transaminase 198 IU/liter (normal, 5-40), glutamate-pyruvate transaminase 91 IU/liter (normal, 0-35), lactate dehydrogenase 558 IU/liter (normal, 190-440). These enzymes soon became normal. On the hospital day 120 the patient underwent tracheostomy and these enzymes were again elevated. At this time CMV was isolated from urine in tissue cultures using HEL cells. Serum IgG and IgM antibodies to CMV were detected by means of ELISA. The patient was then treated with SNMC 50 ml/day (100 mg GL) for 7 days. After GL therapy, liver function became normal, no CMV was isolated from the urine. Her weight gain became normal, and the patient was discharged in good condition on hospital day 150. No side effects were noted during the treatment with SNMC. Although the OKT4/OKT8 ratio and number of OKT4-positive lymphocytes increased slightly, serum Na and K were within the normal range.
Case 2 was a 12-month-old girl with microcephaly and psychomotor developmental delay (Fig. 2). There were no previous problems in the neonatal period, and although weight gain had been slow during breast feeding, the patient had been well. There were no episodes of infection during pregnancy. The patient's mother had IgG antibodies to CMV. Her psychomotor developmental delay became apparent after 3 months of age. When the patient was 8 months old, brain atrophy, subdural effusion and craniosynostosis were diagnosed on the basis of cranial computed tomography at a local clinic. At that time a liver function disorder was detected as a incidental finding. On admission the patient's pulse was 140/min, respiratory rate was 30/min and oral temperature 37.5°C. She weighed 7.9 kg, was 67.8 cm long and had a head circumference of 40.6 cm. The cardio-respiratory examination was normal. The abdominal examination showed no hepatosplenomegaly. The following enzymes were elevated: serum glutamate-oxalate transaminase, 368.1 IU/liter, glutamate-pyruvate transaminase, 232.9 IU/liter, lactate dehydrogenase 344 IU/liter. At the time of admission CMV was isolated from urine by tissue culture. Serum IgG and IgM antibodies to CMV were detected by ELISA. Starting on hospital day 2 the patient received SNMC 50 ml/day for 11 days, three times per week for 2 weeks, twice per week and one a week each for a week. After GL therapy, her liver function became normal and CMV was no longer isolated from her urine. No side effects were noted during therapy with SNMC. The OKT4/OKT8 ratio and number of OKT4-positive cells did not rise and serum Na and K were within the normal range.

Case 3 was a 9-month-old girl with poor weight gain and eczema during breast feeding (Fig. 3). The patient's mother had IgG antibodies to CMV. There were

![Graph showing clinical course of Case 2](image_url)

**Fig. 2. Clinical course of Case 2.** See the legend to Fig. 1 for the symbols.
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no previous problems during the neonatal period. The patient's body weight gain had been slow (3.2 kg at birth, 3.8 kg at one month of age, 5.6 kg at 5 months of age and 6.0 kg at 6 months of age). When she was 6 months old, she was treated for recurrent bronchitis and eczema at a local clinic. At that time liver function disorder was detected as an incidental finding. On admission, the patient's pulse rate was 100/min, respiratory rate 40/min and oral temperature 36.5°C. She weighed 6.7 kg, was 66.2 cm long and had a head circumference of 41.8 cm. Eczema was observed on her face and trunk. The cardio-respiratory examination was normal. The abdominal examination revealed slight hepatomegaly. The following enzymes were elevated: serum glutamate-oxalate transaminase, 322.7 IU/liter, glutamate-pyruvate transaminase, 228.0 IU/liter, lactate dehydrogenase, 600 IU/liter. At the time of admission, serum IgG and IgM antibodies to CMV were detected by ELISA. On hospital day 10, CMV was isolated from the urine by tissue culture. Starting on hospital day 7, the patient was treated with SNMC 50 ml/day for 3 weeks, three times a week for 2 weeks, twice a week for 1 week and once a week for 1 week. Beginning hospital day 56, liver function became normal and no CMV was isolated from the urine. No side effects were noted during the treatment with SNMC. The OKT4/OKT8 ratio and number of OKT4-positive lymphocytes increased slightly, serum Na and K were in the normal range.

**DISCUSSION**

CMV is the most common known cause of congenital viral infection in humans. Only a few infants with congenital CMV infection have classic cytomegalic inclusion disease, and the vast majority of infected infants have no

![Fig. 3. Clinical course of Case 3. See the legend to Fig. 1 for the symbols.](image)
clinical manifestations at birth (Alford et al. 1990). A combination of petechiae, hepatomegaly, splenomegaly and jaundice are the most frequently noted presenting signs. Laboratory findings in patients with symptomatic congenital CMV infection indicate frequent involvement hepatobiliary, immunologic, hematologic, and central nervous system. Perinatal CMV infection refers to infection acquired during the course of delivery as a result of exposure to infected maternal genital secretions or acquired during the postnatal period from ingestion of infected breast milk (Stagno et al. 1980). Although the vast majority of infants with perinatal CMV infection remain asymptomatic, recent reports indicate that this infection may be temporally associated with protracted interstitial pneumonitis and that this form of pneumonitis may give rise to chronic lung disease (Brasfield et al. 1987). In perinatally infected infants, CMV excretion usually begins between 30 and 150 days after exposure to the virus. In such infections, antibody production by the infant usually begins between 4 and 18 weeks postnatally. Three infants investigated in the present study were suspected of perinatal CMV infection. Neonatal and infantile hepatitis are closely related to CMV infection. Active CMV infection indicated by virus isolation and serum IgM antibody response has been described in infants with abnormal liver function tests or hepatomegaly such as three cases in the present study (Chiba et al. 1975; Numazaki and Chiba 1993). It is quite hard to detect inclusion bodies or CMV antigen in the liver tissue from these patients by ordinary immunoperoxidase or immunofluorescence staining (Chang et al. 1992). It was suggested that the abnormal liver function in three cases in this study might be associated with CMV infection. Various antiviral agents have been used in an attempt to treat symptomatic congenital or perinatal CMV infection. These agents have included leukocyte interferon (IFN), IFN stimulators, transfer factor, and nucleotide drugs (idoxuridine, floxuridine, cytosine arabinoside, acyclovir, and ganciclovir). All of these regimens provided little or no clinical benefit and were accompanied by a degree of toxicity unacceptable in the treatment of neonates and infants with CMV infection. Since no adequate studies on the use of ganciclovir or foscarnet (phosphonoformate) for the treatment of congenital or neonatal CMV infections had been conducted, these drugs could not be used for this purpose. In this study, SNMC was administered in a dose of 50 ml/day (GL, 100 mg/day) to three infants with hepatic disorders associated with CMV infection. The liver dysfunction of all three patients improved after the administration of SNMC, and no serious side effects were observed. GL is known as an interferon-inducing agent in vivo, and its in vivo effects may be at least partly mediated by interferon production (Abe et al. 1982). GL also exhibits an inactivating effect on several DNA and RNA viruses in vitro including VZV, HSV, HIV-1, and CMV (Numazaki et al. 1994). In addition, some therapeutic and prophylactic effects on chronic active viral hepatitis have been reported in Japan. GL was administered intravenously to the patients with childhood hepatitis with good effects. Although the exact mechanism of the
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anti-CMV effects of GL have not been clarified, GL is presumed to inhibit the early stages of CMV replication as found in a study of the antiviral effects of GL on VZV (Baba and Shigeta 1987). Further studies, including investigation of the mechanisms of the anti-CMV effects of GL are now in progress.

References