Cytomegalovirus hepatitis and ganciclovir treatment in immunocompetent children

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Ganciclovir treatment in children with cytomegalovirus (CMV) infection is still controversial and only indicated in selected cases. The aim of this study was to evaluate clinical and demographic features of CMV hepatitis in immunocompetent children and to determine the effect of ganciclovir treatment in these patients retrospectively. The study was carried out in a group of 29 children with CMV hepatitis. All the patients were investigated for signs of infection, inborn errors of metabolism, genetic diseases, extrahepatic biliary atresia and other causes of hepatitis. Two patients with congenital CMV infection and two patients with biliary atresia were excluded from the study group. The patients included in the study were divided into two groups: non-cholestatic hepatitis (n=16) as Group I and cholestatic hepatitis (n=9) as Group II. Four (25%) patients in the non-cholestatic group and four (44.4%) in the cholestatic group were treated with ganciclovir for a median of 21 days. The mean age was 9.6±10.9 months (median age 6 months) in Group I, while cholestatic hepatitis patients in Group II were significantly younger, with a mean age of 2.7±0.9 months ($p<0.01$). The most prominent symptoms at admission were diarrhea and vomiting (25%) in Group I. In Group I, all cases (100%) and in Group II, three of four cases (75%) treated with ganciclovir had recovery from acute CMV hepatitis. In the non-cholestatic group, no relapses were observed while one patient in the cholestatic group relapsed and progressed into chronic liver disease. Patients who received supportive treatment showed a marked decrease in GGT, ALT, AST and bilirubin levels spontaneously and no relapses of hepatitis were observed in at least one year of follow-up. Although ganciclovir therapy is not indicated particularly in immunocompetent cases, since most were self-limited infections, in case of progressive and persistent hepatitis, such as in our cases, ganciclovir was a treatment option; no side effect due to ganciclovir therapy was observed in our cases. Although ganciclovir seems to be effective in progressive CMV hepatitis, multicenter randomized studies in a large study group are necessary to determine the efficacy and indications for ganciclovir treatment.

Key words: cytomegalovirus, hepatitis, ganciclovir, cholestasis.

Acute hepatitis in children can be caused by a large number of infectious and non-infectious agents. Many viruses in addition to the primary hepatotropic viruses (hepatitis A-E) should be considered in the etiology of hepatitis that occurs in children[1,2]. The non-hepatotropic viruses account for up to 10% of viral hepatitis and may cause severe liver disease especially in neonates and immunocompromised patients[3]. Some of these relatively common non-hepatotropic viruses are Epstein-Barr virus (EBV), cytomegalovirus (CMV), herpes simplex virus, enterovirus, adenovirus, rubella and parvovirus[2,3].

Cytomegalovirus is a member of the beta-herpesvirus family and commonly infects humans. It is also a leading cause of intrauterine and perinatal infections[4]. Hepatic involvement by this virus may be part of the multiple system involvement or isolated liver involvement such as neonatal hepatitis. This condition was reported to be independent from the presence of cholestasis[2,5].

Although acute hepatitis due to CMV, which is one of the heterophile negative mononucleosis syndromes, is generally mild and benign[6], congenital and perinatal CMV infections can cause progressive liver disease, cirrhosis and even death[7].

The indications for ganciclovir treatment in CMV infections are usually limited to immunocompromised patients, human immunodeficiency virus (HIV) and congenital CMV infections[8,9]. Treatment of CMV hepatitis with ganciclovir in immunocompetent children is still controversial. There is not enough data in the literature concerning the usefulness and side effects of ganciclovir.

The aim of our study was to evaluate clinical and demographic features of CMV hepatitis in immunocompetent patients and to determine the effect of ganciclovir treatment in these patients retrospectively.

Material and Methods

Patients

Twenty-nine patients with CMV-hepatitis were evaluated retrospectively in Hacettepe University, Faculty of Medicine, Ihsan Doğramacı Children's Hospital. The patients were admitted between January 2000 and January 2006. All patients had been investigated for other infectious and non-infectious causes of hepatitis; inborn errors of metabolism, genetic diseases, congenital anomalies such as extrahepatic biliary atresia, and other possible causes were ruled out.

The diagnosis of CMV infection was made by clinical findings, CMV-specific serology and detection of viral DNA by polymerase chain reaction (PCR) in peripheral blood (plasma) and/or urine. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), bilirubin, total protein, albumin, thyroid hormones and alpha-1 antitrypsin levels were also determined. Other infectious agents (hepatitis A, B, C viruses, EBV, herpes simplex 1 and 2 viruses, *Toxoplasma gondii*, enterovirus, rubella, HIV and parvovirus B19) and the other causes of hepatitis were excluded with laboratory tests. Immunodeficiency syndromes were excluded with immunological studies (serum IgG, IgA, IgM, C3 and C4 levels, lymphocyte subpopulations CD3, CD4, CD8, CD56+16 counts). Imaging of liver and biliary tract was done with ultrasonography and liver biopsy was performed in some cases with hepatitis if indicated.

All the patients were admitted to our clinic when they were at least two months of age and since no blood samples were taken in the first three weeks of life, it was not possible to differentiate prenatal, perinatal or postnatal infections. Two of 29 patients had findings of respiratory, ocular (chorioretinitis) and central nervous system (microcephaly, hearing loss and intracranial calcification) involvement in addition to hepatitis. These two patients did not have any history of transfusion. We considered them as congenital CMV based on clinical findings, serum CMV serology, urine and serum CMV PCR, CMV avidity and specific maternal antibodies.

Two patients with cholestatic hepatitis were diagnosed as biliary atresia. These patients and patients with congenital CMV infections with multiorgan involvement were excluded from the study group.

Groups and definitions: The patients evaluated as hepatitis were divided into two main groups according to presence of cholestasis. Sixteen patients without cholestasis were assigned to Group I and nine patients with cholestasis were assigned to Group II.

All patients were breast-fed and no medical problems were identified during pregnancy and labor. Two cases had a history of prematurity and one was small for gestational age (SGA). Two patients in Group I and one patient in Group II had a history of transfusion due to neonatal hyperbilirubinemia. No other organ involvement was detected in any patient.

The patients in Group I (hepatitis without cholestasis) had elevated levels of ALT, AST, and GGT but normal serum levels of bilirubin. Two patients with prolonged fever and massive hepatosplenomegaly in this group had a liver biopsy.

In Group II (hepatitis with cholestasis), patients had increased levels of ALT and AST accompanied by elevation in serum levels of GGT, ALP and bilirubin (≥1.5 mg/dl). Five patients in this group had a liver biopsy.

Definition of CMV infections: CMV infection was diagnosed with detection of CMV-specific IgM and increasing titer of IgG antibodies and positive results of CMV DNA by PCR in blood and/or urine.

Laboratory testing: CMV-specific IgM and IgG was investigated by ELFA (enzyme linked fluorescent antibody) method in automatized system (Vidas, bioMerieux, France) in paired sera at admission and 21 days later in all patients. Nucleic acid was extracted with MagNa Pure Kit (MagNA Pure LC Total Nucleic Acid Isolation Kit, Roche Diagnostics, Germany) and products were amplified with real time-PCR in Cobas Amplicor (Roche Diagnostics, Germany).

Treatment

Vitamin and caloric supplementation (if needed ursodeoxycholic acid in cholestatic patients) was the preferred choice of treatment in patients with hepatitis. Four of nine patients in the cholestatic hepatitis group (44.4%) and four of 16 patients (25%) in the non-cholestatic group were treated with ganciclovir (Table I). Ganciclovir was given as 10 mg/kg intravenous (IV) infusion, two doses for three weeks. No serious adverse effects or complications due to ganciclovir treatment were observed during or after therapy.

Outcome

Biochemical response to the treatment was defined as a marked decrease in serum levels of bilirubin, ALT and AST. Patients in whom CMV-DNA could not be detected by PCR in peripheral blood after treatment were considered to have responded virologically. The recovery of prolonged fever and hepatosplenomegaly was considered as sign of improvement.

Statistical analysis: SPSS 11.5 for Windows (SPSS, Inc, Chicago, IL, USA) was used for statistical analyses.

Results

Sixteen patients had hepatitis without cholestasis (Group I) and nine patients had cholestatic hepatitis (Group II) due to CMV infection. The mean age was 9.6±10.9 months of age

Table I. Summary of Follow-Up of All Patients Treated with Ganciclovir

Patient no.	Sex	Age (m)	Bili& (T/D) mg/dl	ALT& (U)	AST& (U)	Diagnosis	Associated signs	CMV DNA&&	Acute recovery	Follow-up	CMV DNA**	
											I	LT
1	M	7	0.18/0.01	99	102	NCH	PF&HSM	+	+	no relapse	–	–
2	F	30	0.7/0.12	124	145	NCH	PF&HSM	+	+	no relapse	–	–
3	M	3	1.1/0.6	125	212	NCH	HSM	+	+	no relapse	–	–
4	M	4	46.3/15.6	604	687	NCH	HEA&HSM	+	+	no relapse*	–	+
5	M	2	12.5/9.3	209	523	CH	HSM	+	+	no relapse	–	–
6	M	3	12.3/9.3	78	98	CH	HM	+	+	no relapse	–	–
7	M	2	9.5/8.5	131	130	CH	HM	+	+	no relapse§	–	–
8	M	4	11.5/8.3	145	253	CH	HSM	+	–	CLD	+	+

& initial.
&& before treatment in peripheral blood.
** after treatment in peripheral blood.
* no relapse with hepatitis but relapse in 6 months with hemolytic anemia.
§ relapse with recurrent pulmonary disease
Bili: Bilirubin. ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. CMV: Cytomegalovirus. CH: Cholestatic hepatitis. NCH: Non-cholestatic hepatitis.
CLD: Chronic liver diseases. m: Months. I: Immediately. LT: Long term. HEA: Hemolytic anemia. PF: Prolonged fever. HSM: Hepatosplenomegaly. HM: Hepatomegaly.

(range 2 to 42 months, median 6 months) in Group I, while cholestatic hepatitis patients in Group II were significantly younger, with a mean age of 2.7±0.9 months (range 2 to 4 months, median 3 months) ($p<0.01$). There was no sex predominance in either group (Table II).

Although the most prominent symptoms at admission were diarrhea and vomiting (25%) in Group I, jaundice (100%) was the most prominent initial symptom in Group II (Table II). Initial laboratory features are given in Table III.

Four cholestatic patients and four non-cholestatic patients were treated with ganciclovir. Liver biopsy was performed in seven of the patients in both groups whose clinical status worsened during follow-up (2 in non-cholestatic group and 5 in cholestatic group).

Indications and results of treatment with ganciclovir

Group I

Two of the patients treated with ganciclovir in the non-cholestatic group had prolonged fever, and progressive and persistent disease. Fever and hepatosplenomegaly of these patients recovered and urine and blood PCR for CMV turned negative after 21-day ganciclovir therapy. The third patient had no fever but due to the progression of hepatosplenomegaly and progressive increase in liver enzymes, ganciclovir treatment was started and serum levels of GGT, ALT and AST returned to normal limits after two weeks. The fourth case had hemolytic anemia in addition to increased ALT and AST levels and hemolytic anemia responded to ganciclovir treatment within 15 days but relapsed after six months in a self-limited manner. By the end of the therapy, all of the patients had negative results of CMV PCR in blood. These four patients treated with ganciclovir recovered and no relapses with hepatitis were observed during a one-year period (Table II).

Group II

Four patients with persistent cholestatic hepatitis who did not respond to supportive therapy were treated with ganciclovir. By the end of the therapy, three (75%) of them had negative CMV DNA in blood and showed significant decrease in GGT, ALT, AST and

Table II. Demographic Features and Complaints at Presentation in Both Groups

	Non-cholestatic Group I	(%)	Cholestatic Group II	(%)	Total
Cases	16	100	9	100	25
Age	2-42 months (9.6±10.9)	–	2-4 months (2.7±0.9)	–	2-42 months (8.36±10.7)
Girl/Boy	9/7	–	4/5	–	12/13
Blood transfusion history	2	12.5	1	11.1	3
Prematurity (<37 weeks)	0	0	1	11.1	1
SGA	1	6.2	0	0	1
Fever of unknown origin	2	12.5	0	0	2
Jaundice	0	0	9	100	9
Gastroenteritis and vomiting	4	25	0	0	4
Hepatosplenomegaly	7	43.7	7	77.7	14

SGA: Small for gestational age.

Table III. Specific and Non-Specific Laboratory Results of Group I and Group II (comparison of treated and untreated cases) at Presentation

	Non-cholestatic Group I (n=16)		Cholestatic Group II (n=9)	
	Untreated cases (n=12)	Treated cases (n=4)	Untreated cases (n=5)	Treated cases (n=4)
ALT (U/L)	85-696 (224.3 ± 178.7)	99-604 (238.0 ± 244.2)	84-357 (207.2 ± 132.1)	78-209 (140.7 ± 53.8)
AST (U/L)	74-478 (176 ± 109)	102-687 (286.5 ± 270.8)	124-674 (384 ± 249.6)	98-523 (251 ± 193.2)
GGT (U/L)	9-269 (81.7 ± 89.3)	56-444 (187.2 ± 176.8)	124-1021 (537.2 ± 366.5)	209-435 (327.2 ± 101.2)
ALP (U/L)	141-2118 (590.5 ± 532.6)	127-2876 (922.0 ± 1310)	980-2468 (1827.6 ± 646.7)	789-2252 (1575.7 ± 690.8)
LDH (U/L)	435-822 (669.1 ± 126)	347-1393 (766.7 ± 443.4)	513-963 (690.6 ± 180.9)	651-949 (788.5 ± 148)
Total bilirubin (mg/dl)	0.1-0.89 (0.43 ± 0.25)	0.12-46.3 (11.9 ± 22.9)	4.3-14.9 (8.3 ± 4.0)	9.5-12.5 (11.4 ± 1.3)
C. bilirubin (mg/dl)	0.01-0.32 (0.09 ± 0.08)	0.01-15.6 (1.1 ± 3.8)	2.4-11.3 (6.4 ± 3.3)	8.3-9.3 (8.8 ± 0.5)
PT and PTT	normal in all patients	normal in all patients	normal in all patients	normal in all patients
Thrombocytopenia*	0	0	0	1
Anemia**	7	3	2	2
CMV IgM(+)+IgG(+)	12	4	5	3
CMV IgM(+)+IgG(-)	0	0	0	1

*Thrombocytopenia: thrombocyte count below 150,000/mm^3. **Anemia: hemoglobin level below 11 g/dl.
ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. GGT: Gamma-glutamyl transpeptidase. ALP: Alkaline phosphatase. LDH: Lactate dehydrogenase.
C. bilirubin: Conjugated bilirubin. PT: Prothrombin time. PTT: Partial thromboplastin time. CMV: Cytomegalovirus.

bilirubin levels. In one patient with cholestatic hepatitis, CMV DNA in blood persisted and the patient developed chronic liver disease during the two-year follow-up. Three patients had no relapses after their hepatitis recovered with uneventful follow-up (Table I).

Untreated patients in Group I and Group II

Patients treated with supportive treatment showed a marked decrease in GGT, ALT, AST and bilirubin levels spontaneously and no relapses of hepatitis were observed in at least one-year follow-up of these patients.

Discussion

Cytomegalovirus generally causes self-limited, mild and asymptomatic infections in immunocompetent patients. In these patients, CMV infections are characterized as a mononucleosis-like syndrome with fever, cervical adenopathy and elevation in liver enzymes[10]. CMV plays an important role in the etiology of infantile and neonatal hepatitis. CMV hepatitis is relatively common in early ages, especially in early infancy, and in this period is associated with cholestasis[11]. Although not yet confirmed, some authors suggest that CMV could play a major role in development of extrahepatic biliary atresia[12,13]. CMV infections in infancy are important since they might result in cirrhosis and even death[7,14].

In infancy, the biopsy may have features of giant cell hepatitis, with prominent extramedullary hematopoiesis[15]. Cytopathic changes with nuclear and cytoplasmic inclusions may not be obvious in all cases, and additional levels and immunohistochemistry are helpful[2]. Although evidence of CMV infection could not be demonstrated in liver biopsies in our cases, the diagnosis of CMV infection was made by serology and nucleic acid testing in peripheral blood samples. The clinical status of seven patients with hepatitis improved and their nucleic acids became undetectable. According to these results, monitoring virus DNA in peripheral blood by PCR was helpful in follow-up of infection.

In addition to in immunocompromised patients, ganciclovir treatment is suggested in certain severe CMV infections in immunocompetent children[10]. Data on this subject are not obvious enough to state a guideline. There are few

studies concerning ganciclovir therapy in infants and children with CMV hepatitis[16]. The efficacy of this treatment is controversial[17]. All of the patients (100%) in the non-cholestatic hepatitis group and three of four cases (75%) in the cholestatic hepatitis group treated with ganciclovir had recovery from acute CMV hepatitis. This result suggested that ganciclovir could be effective in the acute phase of severe and persistent CMV hepatitis whether associated with cholestasis or not. Ganciclovir was found to be effective in isolated hepatitis patients in terms of recovery of fever and hepatomegaly. Other cases are also reported, such as an immunocompetent girl 17 months of age with prolonged fever and isolated hepatitis successfully treated with ganciclovir, supporting the efficiency of ganciclovir in isolated hepatitis cases[10]. On the other hand, the untreated group (17 cases) showed spontaneous recovery, and this was another point of our study. In this regard, indication for ganciclovir treatment should be restricted to only selected cases with severe and progressive CMV infection.

Although ganciclovir therapy seems to be effective in preventing acute liver failure due to CMV during therapy and just afterwards, no data about the long-term effects of ganciclovir currently exist. Relapse of infection after the cessation of the antiviral drug was observed by other authors as well[16,18]. The patient who did not respond to ganciclovir therapy developed chronic liver disease associated with presence of CMV DNA in the circulation. The infection in the liver tissue was not detected with bioptic technique in any of the patients. This fact leads to the hypothesis that the persisting liver injury in our patient was due to an ongoing immunopathological process, originally triggered by CMV infection, which continued in the absence of the virus in the tissue[17,19]. The relapse of hepatitis with peripheral viremia along with a virologically negative bioptic finding in the liver tissue could be explained by an adjuvant effect of the peripheral viral amplification that enhances the immunopathological liver injury[16]. Although ganciclovir was found to be effective in acute CMV hepatitis, in our study it was found to be ineffective in preventing other long-term complications (Table I).

In conclusion, although ganciclovir treatment in immunocompetent children is still controversial, ganciclovir treatment was correlated with fast recovery of symptoms and findings related with hepatitis in acute non-cholestatic and cholestatic cases.

In our opinion, until the certain indication of ganciclovir treatment is well defined, every patient should be evaluated individually and treatment should be given to those with progressive disease who did not respond to supportive treatment. Multicenter randomized investigations in a large study group are necessary to determine the indications for ganciclovir treatment.

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