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Use of valganciclovir in patients with elevated antibody titers against Human Herpesvirus-6 (HHV-6) and Epstein–Barr Virus (EBV) who were experiencing central nervous system dysfunction including long-standing fatigue

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Abstract

Background: Twelve patients with long-standing symptoms of central nervous system (CNS) dysfunction were found to have elevated antibody titres to human herpesvirus-6 (HHV-6) and Epstein–Barr virus (EBV). All patients had four or more of the following neurocognitive symptoms: impaired cognitive functioning, slowed processing speed, sleep disturbance, short-term memory deficit, fatigue and symptoms consistent with depression.

Objectives: We sought to determine whether elevated antibodies to EBV and HHV-6 indicated chronic viral activation in patients with CNS dysfunction and if their symptoms could be improved by suppressing viral activity with oral valganciclovir.

Study design: Patients with high IgG antibody titers against HHV-6 and EBV who were suffering from central nervous system dysfunction and debilitating fatigue for more than one year (median 3 years, range 1–8 years) were treated with 6 months of valganciclovir in an open label study.

Results: Nine out of 12 (75%) patients experienced near resolution of their symptoms, allowing them all to return to the workforce or full time activites. In the nine patients with a symptomatic response to treatment, EBV VCA IgG titers dropped from 1:2560 to 1:640 ($p = 0.008$) and HHV-6 IgG titers dropped from a median value of 1:1280 to 1:320 ($p = 0.271$). Clinically significant hematological toxicity or serious adverse events were not observed among the 12 patients.

Conclusion: These preliminary clinical and laboratory observations merit additional studies to establish whether this clinical response is mediated by an antiviral effect of the drug, indirectly via immunomodulation or by placebo effect.

1. Introduction

Chronic fatigue syndrome (CFS) is a clinically defined condition characterized by severe disabling fatigue and a constellation of symptoms that prominently feature self-reported impairment of concentration and short-term memory, sleep disturbances, and musculoskeletal pain. Patients suffering from CFS typically experience these symptoms for 6 months or longer. Suggested etiologies of CFS include, but are not limited to: viral or bacterial infections, endocrine-metabolic dysfunction, immunological imbalance, neurally mediated hypotension and depression (Afari and Buchwald, 2003; Fukuda et al., 1994). Most prior studies have found laboratory evidence that EBV and HHV-6 are reactivated more often in patients with CFS than in healthy control subjects or disease comparison groups, but causal inferences have not been made from such an association.

Epstein–Barr virus (EBV) and human herpesvirus type 6 (HHV-6) are enveloped double-stranded DNA viruses belonging to the herpesviridae family. Both viruses are lymphotropic and neurotropic, and both are capable of producing latent infections with immunomodulatory effects (Ambinder, 2003; Ambinder and Lin, 2005; Krueger and Ablashi, 2003). Furthermore, in vitro studies of co-infection with both viruses have revealed that a significant interplay may occur between them (Cuomo et al., 1998; Flamand et al., 1993). The clinical consequence of this interaction remains unknown. However, it has been suggested by various investigators that infection with EBV and/or HHV-6

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may trigger or contribute to the pathogenesis of certain diseases including chronic fatigue syndrome (Bertram et al., 1991; Sairenji et al., 1995) and multiple sclerosis (Hollsberg et al., 2005).

The seroprevalence in the adult population for EBV and HHV-6B is about 90% and most people undergo asymptomatic seroconversion. The high seroprevalence in the general population complicates the interpretation of serological tests in diagnosing reactivation of either virus. Unlike many other viruses, the DNA for EBV and HHV-6 are difficult to detect in either the peripheral blood cells or serum except in cases of primary infection or acute reactivation. Most (Ablashi et al., 2000; Manian, 1994; Natelson et al., 1994; Patnaik et al., 1995) but not all (Buchwald et al., 1996) studies that have examined antibody titers to HHV-6 and EBV in CFS patients have found that there is a significant difference between patients and controls: Manian found 55% of CFS patients had EBV VCA antibodies of 1:320 or above compared to 15% of controls (Horwitz et al., 1985). Sairenji et al. found that CFS patients had elevated antibodies to EBV, HHV-6, human herpesvirus 7 (HHV-7) and ZEBRA (a protein of the immediate early EBV gene BZLF1) compared to healthy controls (Sairenji et al., 1995). Highly elevated early antigen (EA) antibodies are considered indicative of active infection for both EBV and HHV-6. Both Patnaik and Ablashi found elevated antibodies to the HHV-6 EA antibody in CFS patients compared to controls (Ablashi et al., 2000; Patnaik et al., 1995).

HHV-6 and EBV infections can cause immunosuppression and HHV-6 can impair immune response to fungal infections (Cermelli et al., 2006; Sauce et al., 2006; Smith et al., 2005). We suspected that their symptoms could be the result of an immune dysregulation triggered by EBV and HHV-6, especially when reactivated jointly. Alterations in the immune system such as aberrant cytokine profiles have been proposed as the central abnormality in patients with other viruses such as parvovirus B19 (Kerr et al., 2003). Latent EBV and HHV-6 virus can also alter cytokines and induce sickness behavior (Glaser et al., 2006; Yoshikawa et al., 2002).

Valganciclovir is the only known antiviral drug with efficacy against both EBV and HHV-6 that can be administered orally. It has the potential for toxicity, but our experience in using the drug to treat reactivation of viral infections in immunocompromised patients (Gao et al., 2003; Montoya et al., 2001; Montoya, 2004) with minimal adverse effects has made us comfortable with this treatment. If patients are supervised properly, the risk of significant side effects is greatly reduced and antiviral treatment is manageable. We hypothesized that a long course (i.e. 6 months) of valganciclovir could effectively decrease or stop ongoing viral replication of both HHV-6 and EBV and result in a sustained clinical improvement (decrease in antibodies or resolution of lymphadenopathy and fatigue).

2. Materials and methods

2.1. Patients

Twelve patients, ages 21–57 (75% female) were referred to the Infectious Diseases Clinic at Stanford University Medical Center between February 2004 and August 2005 because of their history of chronic fatigue syndrome suspicious for infectious etiology. Patients had been seen by at least five other physicians (range 5–20). Other causes of fatigue had been appropriately excluded. The following laboratory tests were within normal limits in each of the patients who were tried on valganciclovir: complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), alanine aminotransferase (ALT), total protein, albumin, globulin, alkaline phosphatase, calcium, phosphorus, glucose, BUN, creatinine, electrolytes, and urinalysis. All patients met the CDC case definition for CFS (Fukuda et al., 1994) and all had neurocognitive complaints that included four or more of the following symptoms: impaired cognitive functioning, slowed processing speed, sleep disturbance, short-term memory deficit and symptoms consistent with depression. In addition, all had a history of a "flu-like" onset.

2.2. Drug regimen, toxicity monitoring, and fatigue evaluation

Valganciclovir (VGCV) was prescribed as 900 mg twice per day for three weeks followed by 900 mg every day to complete a total of 6 months on the drug. Ganciclovir was approved in June 1989 by the United States Food and Drug Administration for the treatment and prophylaxis of diseases caused by cytomegalovirus (CMV). Ganciclovir has been used at Stanford Medical Center since 1987 for the treatment of several viral diseases observed in immunocompromised patients (Montoya, 2004). A second oral formulation (valganciclovir or L-valine esther of ganciclovir) was introduced in 1991 and achieves serum levels similar to those reached by the intravenous form.

CBC's were followed twice per week for three weeks, followed by once per week for three weeks, and once per month thereafter until VGCV was discontinued. None of the patients in our cohort was taking drugs with known adverse hematological or renal effects.

While on VGCV, patients were instructed to report any new symptoms and on each medical visit they were explicitly asked whether they had experienced fever, chills, unusual bleeding or bruising, infection, unhealed sores or white plaques in mouth, headache, seizures or gastrointestinal symptoms. Pregnancy was not a consideration for any of our patients in the childbearing age; none of them was sexually active. At baseline, and again at each visit, patients were asked to report on their current activity level as a percentage of their pre-illness activity level.

Table 1
Demographic and clinical data on twelve patients with long-standing fatigue and central nervous system dysfunction who received valganciclovir therapy

Patient #	Age	Gender	Flu-like onset	Duration of illness (years)	% of pre-illness activity level		Months on treatment
					At baseline	After treatment	
Responding patients							
1	57	M	Yes	7.5	25	95	6
2	21	M	Yes	7	5	90	6
3	14	F	Yes	1.5	15	90	6
4	48	F	Yes	3.5	15	95	6
5	46	M	Yes	2.5	15	90	6
6	24	F	Yes	1	5	70	6
7	27	F	Yes	1	5	85	6
8	42	F	Yes	8	10	80	3
9	33	F	Yes	1	10	80	6
Median values for responders	33	F	Yes	2.5	10*	90*	6
Non-responding patients							
1	52	F	No	15	25	25	3
2	49	F	No	3	25	25	2
3	28	F	Yes	<1	10	10	3
Median values for non-responders	49	F	No		25	25	3

*p < p = 0.007.

2.3. Serological and molecular testing

In each of the 12 patients, the following serological tests were performed: EBV Viral Capsid Antigen (VCA) IgG and IgM antibodies, antibodies against EBV nuclear antigens (EBNA), antibodies against EBV early antigens (EA), HHV-6 IgG and IgM antibodies and CMV IgG and IgM antibodies (Focus Diagnostics, Inc., Cypress, CA, USA). HHV-6 and EBV testing was done by IFA and CMV tests were done by ELISA. The HHV-6 kits were purchased at Panbio (Columbia, MD) and the EBV kits are FDA approved kits sold by the Products division of Focus Diagnostics. At initial evaluation, each of the 12 patients had a polymerase chain reaction (PCR) test performed in serum for the following viruses: EBV, HHV-6, and CMV.

2.4. Statistical testing

To compare EBV and HHV-6 serologic titers before and after valganciclovir therapy among the 12 patients, paired non-parametric tests were performed (Sign test). To compare demographic and serologic variables between those who responded to valganciclovir and those who did not, the Mann–Whitney test was used. Statistical analysis was performed using the Epi Info Version 3.3.2.

3. Results

As shown in Table 1, of the 12 patients, 9 "responders" had a dramatic improvement in their fatigue and central nervous system symptoms ($p = 0.007$) and 3 "non-responders" failed to report any progress. Central nervous system symptoms such as "brain fog" and other cognitive abnormalities were among the first symptoms to improve. Most improvement of clinical symptoms occurred between weeks 6 and 12. In the first several months, significant improvement was observed in physical activity at home. Subsequently, each of the responders was able to return to work or full time activities. The new level of "near-normal" activity has been sustained for greater than 9 months (up to 31 months) after discontinuation of the drug at week 24 in each of the nine patients.

The mean age of the responders was 35.4 years (range: 14–57) and that of the non-responders 43 years (range: 28–52). Of the 9 responders, 9 (100%) experienced the onset of their chronic fatigue syndrome as a "flu-like" illness and 4 (45%) developed lymphadenopathy. Whereas of the 3 non-responders, only 1 (33%) had a "flu-like" illness at the onset of the disease and 1 (33%) developed lymphadenopathy. The activity level of both responders and non-responders was severely compromised, compared to pre-illness activity level, at baseline (10% in responders, 15% in non-responders).

All of the nine responders experienced an initial worsening of their already severe symptoms. This worsening occurred between weeks 2 and 4, and was severe enough to make several patients stay in bed for several weeks. In one patient where the WBC differential data was collected, this worsening period coincided with a 25% drop in WBC count

Table 2
Antibody titers against HHV-6 and EBV antigens at baseline and after valganciclovir therapy

Patient #	HHV-6 IgG		EBV VCA IgG		EBV EBNA Ab		EBV Early Ag (R+D) IgG	
	Pre-Rx	Post-Rx	Pre-Rx	Post-Rx	Pre-Rx	Post-Rx	Pre-Rx	Post-Rx
Responding patients								
1	320	320	5120	640	640	160	640	160
2	1280	160	10240	5120	160	40	640	640
3	1280	320	640	160	160	320	160	320
4	2560	2560	1280	640	40	40	160	160
5	640	320	1280	640	640	320	640	640
6	640	160	2560	320	160	40	160	160
7	320	640	2560	640	10	40	160	40
8	2560	320	2560	640	10	0	640	640
9	1280	2560	640	160	40	10	80	20
Median values for responders	1280	320	2560	640	160	40	160	160
Non-responding patients								
1	40	ND	160	160	40	40	160	160
2	80	ND	320	320	80	80	ND	ND
3	2560	ND	640	ND	160	ND	160	ND
Median values for non-responders	80	ND	320	240	80	60	160	160

and an 80% drop in monocytes. The three non-responders did not experience this worsening in their symptoms.

None of the 12 patients experienced any side effects to VGCV that required its discontinuation or experienced abnormalities in their laboratory tests including clinically significant hematological toxicities.

IgG antibody titers against HHV-6 and EBV antigens at baseline and after valganciclovir therapy for the responders and non-responders are shown in Table 2. In responders, the median HHV-6 antibody titer dropped from 1:1280 to 1:320 ($p = 0.271$) and the median EBV VCA titer dropped from 1:2560 to 1:640 ($p = 0.008$). Two of the 3 non-responders had no significant change in their titers against EBV antigens after VGCV therapy and values were not obtained for the third. Two of the non-responders did not have elevated antibody levels that meet our current threshold for treatment, but had other evidence of a viral syndrome.

Of the 12 patients in our cohort, only one patient had a positive IgM for HHV-6 (patient #8). The 12 patients were negative for EBV VCA IgM antibody. Neither EBV, HHV-6 nor CMV DNA was detected by PCR in the serum; assessment of viral DNA on whole blood was not performed. Seven of the patients had high HHV-6 antibody titers (patients #2–6, 8, 9), five had elevated antibody titers to EBV (#1, 2, 6–8), and three had elevated antibodies to both viruses (#2, 6, 8).

Reduction in IgG antibody titers, duration of fatigue and level of activity change in 9 patients whose clinical improvement was associated with the use of valganciclovir are shown in Table 1. In three of the nine responders the EBV EA antibody dropped by four-fold or more. EBNA antibodies dropped in six of the nine responders. Of the 9 responders, 4 (45%) were positive for CMV IgG and 1 was positive for CMV IgM. Of the 3 non-responders, 2 were CMV IgG positive. Only one patient was positive for CMV IgM antibodies (patient #8).

4. Discussion

This was an open-label study of valganciclovir (VGCV) in 12 patients with CFS and elevated antibody titers to both HHV-6 and EBV. VGCV treatment was associated with reduction in antibody titers in most patients. A majority of the treated patients also had dramatic improvement in activity level, although three did not. Obviously, a placebo effect is always possible in open-label studies. Many previous treatments had been tried in these 12 patients, without any benefit, suggesting that they were not placebo responders. Moreover, patients with CFS appear to be less likely than patients with other diseases to experience a placebo effect (Cho et al., 2005). Nevertheless, large randomized trials are essential before VGCV therapy is given routinely in patients with CFS and elevated antibody titers to HHV-6 or EBV. Based on our experience, such studies should probably be restricted to CFS patients whose illness began with an acute flu-like illness, which may be a minority of patients with CFS in the community at large (Solomon and Reeves, 2004).

Since HHV-6 is typically acquired by the age of two and EBV is generally acquired in early childhood or as a young adult, we interpreted the elevated titers as a sign of reactivated rather than primary infection. The antibody titers of the patients were considerably higher

than those in 12 healthy controls, tested in the same laboratory. However, reference values for antibody titers against HHV-6 and EBV do vary by laboratory and variations of 1–2 dilutions in results on tests run on the same sample are considered common in most commercial laboratories. Focus Diagnostics, the laboratory used by Stanford for the EBV, HHV-6 and CMV serological tests, has a reference range that is higher than what has been reported in the literature (Savoldo et al., 2002; Straus et al., 1985; Tobi et al., 1982). At our request, Focus Diagnostics tested 12 healthy controls and the median titers were 1:80 for HHV-6 IgG and 1:320 for EBV VCA IgG. When fifty laboratory workers were tested at Focus, median values were slightly higher: 1:160 for HHV-6, and 1:640 for EBV VCA.

Although EBV EA antibodies dropped in three responders, it did not drop in the others, which was contrary to our expectations. Since antibody levels fall slowly over time, these results may understate the full extent of the drop. Antibody measures cannot determine whether a patient has an active viral reactivation. However, the fact that we observed falling EBV and HHV-6 titers concurrent with clinical recovery is significant. Larger studies and standardized assays are necessary to determine how useful these serological assays can be in identifying prospects for treatment. Molecular assays available in clinical laboratories were not informative. None of the responding patients was positive for HHV-6 or EBV DNA using an assay capable of detecting as few as 200 copies/ml. DNA of these viruses is typically detected only during a primary or acute infection. Better biomarkers are needed to identify reactivation of chronic disease and assess response to treatment.

Both HHV-6 and EBV can reactivate in a healthy person in response to an acute illness and normal controls can be found with elevated antibody titers to HHV-6 and EBV, especially in those under the age of thirty who are more likely to have had a recent primary infecton. This makes the assessment more complex and underscores the necessity of a careful evaluation by an experienced clinician, in order to differentiate between transient reactivation due to other illness, asymptomatic elevation of viral titers, and a chronic reactivated viral infection which would deserve treatment.

Clinical evaluation and a history suggestive of a viral etiology must remain the primary determinant for treatment. Treatment cannot be based solely on antibody levels or detection of viral DNA. In cases where humoral immune response is impaired, antibody levels may not be elevated at all or only slightly elevated above the mean. Also, there may be persistent viral infection in the CNS tissue with no trace in the peripheral blood. A prospective placebo controlled trial of valganciclovir is necessary to determine whether a subset of CFS patients would benefit from treatment. The fact that antibody titers to either HHV-6 or EBV dropped four-fold or more in several of these patients suggests (a) some if not all of these patients had active infections and (b) elevated antibody levels can be useful to confirm a suspicion of a chronic infecion that has reactivated.

In this study, treatment with oral VGCV did not produce any serious adverse effects, but it was not without some difficulties. As noted above, all of the responding patients reported some degree of worsening of their condition during their inital month of treatment. This worsening resembled a Jarisch–Herxheimer-like reaction and included several days of myalgias, chills, headache, worsening of the "brain fog" and fatigue, and skin rash in some patients. The pathology of this response is unclear, but may be mediated by an immune response to transiently increased circulating viral antigen(s).

Adverse events for patients using VGCV is far higher for immunocompromised patients including those with AIDS-associated CMV retinitis and solid organ transplants with CMV disease. Neutropenia was reported to be 27% for retinitis patients (Roche Valcyte product information: (http://www.rocheusa.com/products/valcyte/) and 8% in solid organ transplants (Paya et al., 2004). In this small study, none of our patients experienced any hematological adverse effects or untoward reactions requiring the discontinuation of the VGCV.

Several other limitations of this study need to be acknowledged. We did not test for HHV-7 or distinguish between the HHV-6 A and B subtypes. Also, despite the fact that the improvements in physical activity and cognitive functions were dramatic and impressive, they were self-reported and not objective measurements. We are planning a double-blind placebo controlled trial with more comprehensive evaluation of the functional status of subjects, using established and validated instruments as well as better documentation of viral activity.

In summary, the results of this preliminary study suggest that a subset of patients with CFS and elevated antibody levels to HHV-6 and EBV may have an illness that is caused by reactivation of these viruses, and that is responsive to VGCV antiviral therapy. Further definition of this subset of patients with better and more standardized viral assays is required, as are large randomized controlled trials with long-term follow-up to confirm the possible value of antiviral therapy in patients with CNS dysfunction and symptoms of sustained fatigue. In addition, we propose a new medical term for the syndrome that may best describe our patient cohort: Virus Induced CNS Dysfunction. It may be applied to the subset of CFS patients who have elevated HHV-6 and EBV titers and clinical symptoms of a viral syndrome with neurocognitive complaints and sustained fatigue. Other viruses may also play a role in defining additional CFS patient sets.

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