Ribavirin and Interferon-α for the Treatment of Patients with Chronic Fatigue Syndrome Associated with Persistent Coxsackievirus B Infection: A Preliminary Observation

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ABSTRACT

Several studies have demonstrated elevated neutralizing antibodies to coxsackievirus B (CBV) and the presence of enteroviral RNA in peripheral blood in a subset of patients with chronic fatigue syndrome (CFS). Of 10 patients with stable, persistently high titer of neutralizing antibody against CBV-3 and CBV-5 were treated with ribavirin for 4 months, and 5 patients were subsequently treated with interferon/ribavirin for 2 to 6 months. Seven of 10 patients had significant improvement of fatigue and viral symptoms during ribavirin therapy, along with more than a fourfold decrease of neutralizing antibody. Most patients relapsed within 1 to 2 weeks of medication discontinuation, with subsequent rebound of neutralizing antibody to pre-treatment baseline and had detectable enteroviral RNA in blood leukocytes. Combination therapy resulted in the disappearance of enteroviral RNA, decrease in neutralizing antibody in 4 of 5 patients, and significant, short-term symptomatic improvement following completion of therapy in 4 of 5 patients. Relapse occurred about 4 months later in most patients along with rebound of neutralizing antibody titer and reappearance of enteroviral RNA. Antiviral therapy may be beneficial in a subset of CFS patients with persistent CBV infection.

INTRODUCTION

A severe flu-like illness that occurred in the majority of cases of chronic fatigue syndrome (CFS) followed by persistent fatigue suggests an infectious etiology triggers or causes this syndrome.[1] Endogenous DNA viruses such as Epstein-Barr virus (EBV) and Cytomegalovirus (CMV), Parvovirus B 19, and *Chlamydia pneumoniae* have been reported to cause prolonged fatigue, fevers, and many other symptoms of CFS in small subsets of patients,

and many of these patients respond to specific anti-infective therapy.[2-4]

The role of enteroviruses as the causative agents of CFS has been controversial. A number of studies have detected enteroviral RNA in the muscle biopsies and blood of CFS patients.[5-6] Galbraith et al added to this finding by performing phylogenetic analysis of the amplified cDNA from the blood of patient; the sequences closely matched those found in coxsackievirus B (CBV) and echoviruses.[7] Cunningham also demonstrated that persistence of enteroviral RNA in patients with CFS was associated with abnormal production of equal amounts of positive and negative strands of enteroviral RNA.[8] The latter was confirmed by Tam and Messner, in a murine model of CBV induced myositis, as the mechanism of enteroviral persistence through the production of a double-stranded RNA complex.[9] Recent in vitro experiments also demonstrated that enteroviral persistence and reactivation are dependent on cell cycles.[10]

Our recent studies also demonstrated the presence of enteroviral RNA in the blood mononuclear cells, confirmed by sequence analysis of 7 samples, but not in the plasma of CFS patients by 2 different enteroviral RNA assays.[11] A recent larger study also demonstrated the presence of enteroviral RNA, on repeat testing, in 92 (39 %) of 236 patients with CFS as compared to 2 (4%) of 52 controls.[12]

Interferon and ribavirin were effective agents against CBV in carrier cultures.[13] Two small clinical studies have demonstrated efficacy of interferon in CFS patients but the follow-up was only 3 months; and furthermore, none of the patients were defined virologically.[14,15]

In this report, CFS patients thought to have chronic CBV infection were treated with ribavirin alone and with the combination of ribavirin and alpha-interferon. During and after completion of therapy, the antibody levels decreased, only to increase again when the patients relapsed off therapy, along with reappearance of enteroviral RNA in most patients.

MATERIALS AND METHODS

Patients

Ten of the most symptomatic patients who fulfilled the 1994 CDC criteria for CFS were selected on the basis of continuous debilitating symptoms and persistently high levels of neutralizing antibody for CBV-3 or CBV-5 over a 1 to 2 year period. Eight females (aged 21-42) and 2 males (aged 18, 37) were given medications. Patients gave written informed consents for the various phases of treatment and viral study. The investigation on the presence of enteroviral RNA in blood was approved by the IRB of UCLA-Harbor medical center, Torrance, Calif. One hundred fifty controls from the community also had antibody testing for CBV. The mean and standard deviation for CBV-3 and CBV-5 were 12 ± 16, 14 ± 22, respectively. The patients were considered to have chronic CBV infection, if the titers were consistently greater than 4 times the mean + 2 standard deviations of the controls.

Laboratory Investigation

Antibody for CBV was performed by ARUP laboratory, Salt Lake City, Utah, using the microneutralization method. Serial specimens were not performed in parallel, but internal controls of known titers were utilized at the reference laboratory for the neutralization assay, and the inter-assay variation was ≤ 2 folds or the test was rerun.

Enteroviral RNA determination by Hybridization-capture method

The peripheral blood leukocytes (PBL) were harvested from 1 mL of whole

blood, within 2 to 4 hours of blood draw, and the RNA extracted using the Qiagen blood mini kit. The RT-PCR was performed using Qiagen one-step RT-PCR enzyme kit and the biotinylated primer set from Chemicon enteroviral oligodetect kit. The manufacturer's instructions were followed except that the RT step was performed at 55°C. The final optical density was measured in an EIA reader at 440 nm after the streptavidin-horse radish peroxidase and TMB/E steps. The sensitivity of the assay, as determined by an enteroviral RNA standard (Ambion, Austin, Tex) varied between approximately 80 to 800 copies of RNA per mL of whole blood. A positive sample was defined as final O.D. of ≥ 1.00 when 10 copies of enteroviral RNA standard yielded a value ≥ 1.0. Very minute amounts of viral enteroviral RNA (approximately 80 to 800 copies per mL of blood) were found in the PBL of these patients and only few patients had constantly positive enteroviral RNA in PBL on serial sampling.

Treatment

Four hundred mg ribavirin was given 2 times a day with food for at least 1 month. The patients who had substantial improvement of symptoms were treated for a total of 4 months. Ribavirin was discontinued in patients who did not respond by the end of one month. None of the patients have responded to prior treatment with antidepressants, stimulants, Acylovir or Azithromycin.

Combination therapy, Interferon α-2b (Intron-A, Schering-Plough) 3 million units was administered subcutaneously 3 times a week, along with 400 mg ribavirin, twice a day, to 5 patients who were enteroviral RNA positive and continued to have high titers of neutralizing antibody for CBV. Only two patients (A and C) tolerated 6 to 8 weeks of therapy. The duration of treatment for patients B, D, and E was 4, 6, and 4 months, respectively.

Clinical Criteria for Improvement

The energy index (EI), a self-assessment scoring system, described by Lerner[3] was used to assess improvement of energy level. All of the patients had EI ≤ 4 out of a 0 to 10 scale before treatment. Clinical improvement was defined as resolution of low-grade fevers, night sweats, myalgia or cognitive dysfunction, and improvement of energy level by at least 3 points based the energy index.[3]

RESULTS

Seven patients had marked improvement of fatigue and other flu-like symptoms within 2 to 3 weeks of taking ribavirin. Mild headaches and nausea were the most common side effects. Three patients had an 11% to 15% reduction of hemoglobin at the end of the second month, but were able to continue the medication without dose modification. Three of the 7 patients (B, C, and D) started to deteriorate before the end of the 4-month treatment period, although all 3 were still better than baseline when the treatment was completed. Six patients relapsed with recurrence of all of the symptoms of CFS within 1 to 2 weeks of drug discontinuation. All seven patients had a fourfold or greater reduction of neutralizing antibody during treatment. However, the levels rebounded at the end of treatment in 3 patients (B, C, and D), who deteriorated before the end of treatment, and in all other four responders after the treatment was completed (Figure 1). Patient E relapsed 3 to 4 months following therapy along with a delay rise of the antibody titer. Three patients did not respond to 1-month treatment with ribavirin, and the antibody titer remained elevated at 1:640 (normal ≤ 1:10) or decreased ≤ 2 folds

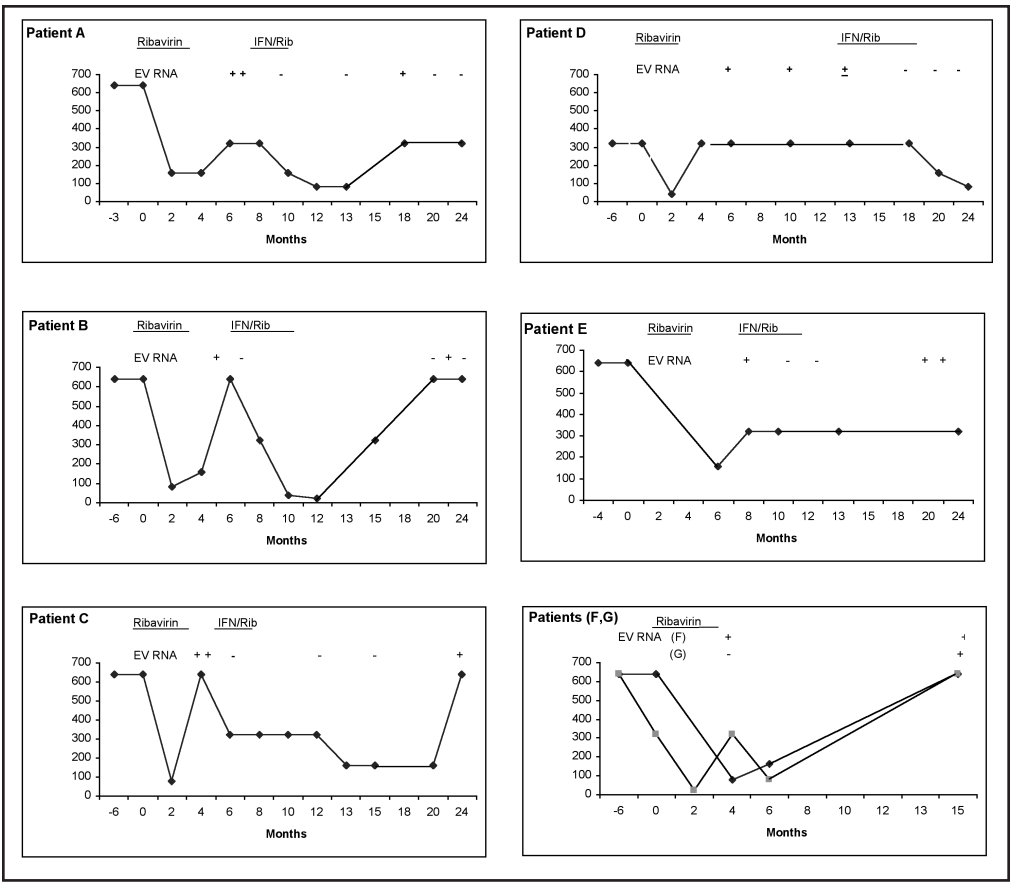

Figure 1. Change of neutralizing antibody titers to coxsackievirus B and the detection of enteroviral RNA in the peripheral blood leukocytes of patients (A-G) with chronic fatigue syndrome during and after treatment with ribavirin ± interferon α-2b. Titers of neutralizing antibody for CBV-3 or CBV5 were measured before, during, and after each treatment. Ribavirin treatment was 4 months and the combination of interferon and ribavirin treatment was 2 to 6 months, as indicated by the underline. Enteroviral RNA detection was performed on peripheral blood leukocytes on all of the patients after the ribavirin treatment, and indicated as positive (+), negative (-) or ± (the optical density reading was between 0.5 to 1.0).

over the next 6 to 12 months (data not shown).

The enteroviral RNA assay became available after the patients finished the ribavirin treatment. Enteroviral RNA was found in PBMC of all 5 patients and on 2 consecutive specimens, obtained 2 weeks apart, for 2 of 3 patients prior to the start of the combination therapy. Enteroviral RNA became negative 2 to 8 weeks after the start of therapy in all 5 patients (A to E). Fatigue and flu-like symptoms did not improve, and actually worsened in all of the patients while receiving interferon and ribavirin. Two patients (A, C) could only tolerate 2 months of therapy. One patient had significant depression and was placed on a low-dose antidepressant. About 2 to 4 weeks following the completion of therapy, 4 of 5 patients felt significantly improved; and the antibody titers decreased for at least several months. However, relapse occurred in 3 of 4 responders about 4 to 5 months post-treatment, after vigorous exercise in 2 patients (A and B). The antibody level rebounded in 3 patients (A, B, and C)

and the enteroviral RNA became positive at least once in all three patients who relapsed. One patient (D) who was treated with combination treatment for 6 months remained much improved at 6 months follow-up, but no further follow-up was possible. The titer of neutralizing antibody remained low and the enteroviral RNA was still negative at 6-months post treatment. Patient E did not respond to the combination therapy; and the antibody level remained the same over the next $1^1/_2$ years. Viral RNA was positive at least once following discontinuation of combination therapy.

Patients F and G, chose not to have combination treatment; and the neutralizing antibody rose to equal to or higher than pre-treatment levels at the 15-month point. Patient F had positive enteroviral RNA in PBL right after ribavirin therapy, and both patients had detectable enteroviral RNA in PBL on subsequent follow-up.

DISCUSSION

All of the patients had persistently elevated antibody levels for at least 6 months prior to treatment, and none of the patients had spontaneous symptomatic improvement or change of antibody titers in response to conventional therapy. With ribavirin treatment, the decrease of antibody titers correlated with symptomatic improvement in all 7 patients, and subsequent increase of antibody titer, although much slower over time in some patients, correlated with relapse. Enteroviral RNA was positive in all 5 patients before the combination treatment, which rapidly disappeared with treatment, only to turn positive, at least transiently, when the patient relapsed months after the discontinuation of therapy. The decline in neutralizing antibody titers and disappearance of enteroviral RNA with an effective therapy was also demonstrated in a previous treatment study on CBV myocarditis.[16]

Potential weakness of these observations included the inter-assay variability of the neutralizing antibody test since the tests were not run in parallel. Although an internal standard was used for each run at the reference laboratory, the possibility existed for greater than twofold variation of the antibody test for different runs. During ribavirin therapy, however, the reduction of antibody titers was greater than fourfold for all of the responders; and the antibody titers remained fairly constant off therapy for a number of the patients. These data would argue against a spontaneous decrease of antibody titers and support a good correlation between therapeutic response and decrease of antibody titer. On the other hand, patient C was still quite symptomatic after she relapsed, 4 months after the combination treatment, but the antibody titers remained low for at least 6 months before a rebound. Secondly, none of the responders felt completely normal after treatment, although they were significantly improved as defined by the response criteria. Unfortunately, there are currently no objective tests of fatigue that would validate the subjective complaints. Thirdly, the enteroviral RNA was not consistently found in the blood after the patient relapsed. Although diagnostic of persistent infection, enteroviral RNA was not constantly positive even in the sickest CFS patients, and was found uncommonly in patients with milder illness.[12]

Previous research done on subset of the CFS patients and in animal models clearly implicated an abnormal mechanism of RNA replication in chronic enteroviral infection.[8,9] Since active virion production is possible only when the concomitant synthesis of VP1 capsid protein is linked to RNA synthesis,[17] a "stalemate" situation is probably created

in chronic enteroviral infection. In immunocompetent patients, initial inflammatory response to acute enteroviral infection, including but not limited to interferon production, decreases protein synthesis of infected cells and causes an accumulation of positive and negative RNA strands, which eventually form a stable double-stranded RNA complex.[9] Viral persistence may occur in different types of cells including long living, tissue macrophages-cells responsible for uptake of virus and cellular debris, as demonstrated in histochemical study of virus-infected murine heart.[18] Double-stranded RNA is a potent inducer of pro-inflammatory cytokines and interferon,[19] which further perpetuates viral persistence by inhibiting protein synthesis and may be partly responsible for the symptomatology of CFS. This type of mechanism of viral persistence reconciles the 2 seemingly opposing observations for the past 2 decades: absence of live virion in chronically infected patients and animals, and the finding of enteroviral RNA in the blood or other tissues.

Ribavirin is incorporated by RNA-dependent RNA polymerase (RdRp) of RNA viruses, which can cause lethal mutagenesis of poliovirus in cell culture.[20] The breakthrough observed in 3 of 7 patients and eventual rapid relapse of symptoms in all 7 patients associated with isolation of viral RNA in PBL suggest inadequate suppression of RNA replication. Subsequent combination therapy was followed by longer remission, about 4 months on average, as compared to 1 to 2 weeks after monotherapy with ribavirin, although symptomatic relapse was associated with reappearance of viral RNA in blood. Taken together, these observations suggest that low-grade RNA replication may indeed occur in these patients.

The response to ribavirin and interferon is consistent with the previous report of symptomatic improvement after 3 months of interferon treatment.[14,15] From the current study, it is apparent that all of the treated patients relapsed after 3 months of observation, whereas the previous report only followed the patients for up to 3 months. At least 1 to 2 years of follow-up is needed in these treated patients in order to define the short- and long-term benefit of any therapy.

These results suggest that chronic CBV infection causing symptoms of CFS may be amenable to antiviral therapy. The change of neutralizing antibody titer to CBV may be a good marker of therapeutic response. Although, a specific marker of persistent CBV infection, enteroviral RNA is not detected consistently enough in serial samples to be a useful test to monitor the effect of therapy. In the future, controlled trials with newer interferon preparations and ribavirin or other RdRp inhibitors will be needed to define the true benefit of antiviral therapy.

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