ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1984, p. 25–28
0066-4804/84/010025-04$02.00/0

Vol. 25, No. 1

Alpha-Interferon Administration in Cytomegalovirus Retinitis

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Received 9 May 1983/Accepted 17 October 1983

Four patients, including three with the acquired immunodeficiency syndrome (AIDS), were treated with high-dose, buffy coat-derived alpha-interferon for progressive cytomegalovirus retinitis. Two of these patients had decreased viruria during therapy and the other two had increased viruria. There was evidence of progression of disease despite therapy in all patients, although the retinitis eventually became quiescent in the patient without AIDS. The severe immunosuppression encountered in AIDS patients complicates the management of cytomegalovirus and other opportunistic infections.

Antiviral therapy of systemic cytomegalovirus (CMV) infection remains a difficult problem. Various drugs have been used for CMV infection in uncontrolled settings, with inclusive results. Vidarabine may suppress viral excretion during therapy, without effect on clinical manifestations (6), or with possible clinical benefit when given in high, near-toxic doses in CMV retinitis (10). Acyclovir was reported to have some clinical benefit in immunosuppressed hosts with CMV infection in one institution (2), but in another study, seven of eight bone marrow transplant recipients with biopsy-proven CMV pneumonia died despite high-dose acyclovir therapy (12). Systemic leukocyte interferon temporarily suppressed viruria in infants with congenital CMV infection (1). Bone marrow transplant recipients with CMV pneumonia did not demonstrate clinical improvement from treatment with leukocyte interferon alone (9) or in combination with vidarabine (8).

CMV retinitis has become more commonly recognized as a complication of chronic CMV infection in immunosuppressed hosts, including those with the acquired immunodeficiency syndrome (AIDS) (5). Patients with CMV retinitis are good candidates for antiviral trials for several reasons. The disease tends to evolve less rapidly than some other CMV syndromes, thus allowing time for virological confirmation of the diagnosis before initiating therapy and time for a therapeutic effect to have an impact on the disease process. Because the eye lesions are characteristic and easily followed by objective criteria such as fundus photographs, clinical response may be readily assessed. Also, the risk of permanent visual loss and the lack of proven effective therapy has stimulated the use of experimental antivirals in an effort to preserve vision.

We report here our experience in the treatment of four patients with CMV retinitis, using high-dose human leukocyte interferon. Three of the patients had AIDS. We monitored response clinically and virologically, using traditional tissue culture methods as well as the recently developed DNA hybridization assay for rapid measurement of viruria (3).

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CASE REPORTS

Clinical features and virological data from the four treated patients are summarized in Table 1.

Case 1. A 36-year-old homosexual male was referred for interferon treatment of CMV retinitis after experiencing myalgias with vidarabine therapy. He had a 2-year history of Kaposi's sarcoma first manifest as an oral lesion. Subsequently, he developed generalized lymphadenopathy and splenomegaly. He was treated with vinblastine and then with doxorubicin and vincristine. His last chemotherapy was 4 months previously. At that time he developed *Pneumocystis carinii* pneumonia, which was treated with trimethoprim-sulfamethoxazole and pentamidine. Other problems included oral candidiasis, chronic diarrhea, perianal herpes simplex infection, and leukopenia. Two months before referral, visual loss in the left eye was noted, and a diagnosis of CMV retinitis was made. Viral culture of the urine showed CMV. Because of progression of lesions, a course of vidarabine was attempted, but this caused leukopenia and myalgias, and therapy was stopped after 2 weeks. Two weeks later, after resolution of the myalgias, interferon was begun at a dose of 5×10^6 U per day. Pretreatment urine culture showed 3.9 log $TCID_{50}$ per 0.2 ml of CMV, with a positive DNA hybridization assay. A buffy coat culture was positive for CMV on day 2 of treatment. During therapy, granulocyte counts fell from 1,300/mm^3 to a range of 700 to 1,000/mm^3. The interferon dose was nevertheless increased to 5×10^6 U twice daily beginning 12 days after beginning therapy. This did not result in any further decrease of granulocyte counts; in fact, they rose to pretreatment levels. The patient experienced moderately severe anorexia and malaise, which appeared worse than that present before treatment. The increased interferon dosage was given for 26 days (for a total of 38 days of interferon). Four-hour post-injection interferon levels in the blood ranged from 80 U/ml at the lower dose to 225 U/ml at the higher dose. Viruria increased during treatment as measured by culture and DNA hybridization. Eye lesions gradually progressed during the treatment period. After discontinuation of treatment, the patient was not available for follow-up examination, but his disease resulted in total blindness, and he died 2 months later from Kaposi's sarcoma.

Case 2. A 43-year-old homosexual male was referred for interferon treatment because of a 3-week history of visual loss due to CMV retinitis. AIDS had been diagnosed 10

TABLE 1. Clinical and virological features of leukocyte interferon-treated CMV retinitis patients

Feature[a]	Case 1	Case 2	Case 3	Case 4
Age (yrs)	36	43	24	46
Underlying disease	AIDS	AIDS	AIDS	Hodgkin's disease
Interferon daily dosage (10^6 U)	5–10	20–36	10	10
Duration of therapy (days)	38	25	19	62
Serum interferon levels (U/ml, range)	80–225	78–232	87–109	60–84
Viruria, measured by:				
Tissue culture (log $TCID_{50}$ per 0.2 ml)				
Pretreatment	4.2	6.9	0.9	6.9
Wk 1 of therapy	≥5.2	4.5	1.5	5.2
Last wk of therapy	6.9	4.2	2.5	4.2
DNA hybridization (log cpm/10 ml)[b]				
Pretreatment	3.4	4.2	2.5	4.7
Wk 1 of therapy	4.3	3.4	2.5	5.2
Last wk of therapy	3.8	3.4	2.7	3.5
Status of retinal disease posttreatment	Worse	Worse	Worse	Inactive

[a] All patients were males.
[b] Positive for CMV when log cpm is greater than 2.8.

months previously when the patient developed *P. carinii* pneumonia and manifested the typical reversal of the T cell helper/suppressor ratio. Six months later he had a severe episode of herpes zoster with cutaneous dissemination. Thereafter, he remained easily fatigued and had recurrent episodes of oral and genital herpes simplex infection. At referral, ophthalmological examination revealed extensive bilateral involvement with chorioretinitis typical of CMV, with imminent threat to vision because of lesions surrounding the macula and optic nerve in both eyes. The urine was positive for CMV by culture and DNA hybridization; a buffy coat culture for CMV was also positive. Interferon was begun at 10×10^6 U twice daily, escalating after 1 week to 15×10^6 U twice daily, and finally to 18×10^6 U twice daily at 3 weeks for 5 additional days at this dosage. Total treatment duration was 25 days. Interferon level in the blood was 45 U/ml before the patient received any therapy. Four-hour post-injection interferon levels reached 232 U/ml at the highest dosage. During this time, granulocyte counts remained stable in the range of 2,500 to 3,500/mm^3, and platelet counts were normal. The patient experienced severe anorexia, fatigue, and malaise at the highest dosage of interferon, and more moderate symptoms at the lower dosages. Fever was present before and during therapy; this was controllable with antipyretics. Other than systemic CMV infection and interferon, no other source of fever could be identified. A high-grade viruria recorded at the beginning of therapy by both culture and DNA hybridization decreased to a moderate viruria which persisted during the entire treatment period. Viremia was detected on cultures of peripheral blood leukocytes done on days 3, 7, and 16 of therapy. During the first 2 weeks of treatment, eye examination showed relatively unchanged findings, but considerable progression was noted at the conclusion of therapy, resulting in decreased visual acuity bilaterally.

Case 3. A 24-year-old homosexual male was referred for evaluation because of a 2-week history of progressive visual loss in the right eye. Three months earlier he had been hospitalized for treatment of *P. carinii* pneumonia. He also had oropharyngeal candidiasis. At that hospitalization, lung specimens did not reveal CMV on culture, but the virus was cultured from the urine of the patient. A diagnosis of AIDS was made. At this referral, funduscopic examination revealed acute chorioretinitis of the right eye with an appearance typical of CMV infection. Comparison of fundus photographs taken 10 days apart showed progression of the disease over the interval. Virus was cultured from a urine specimen at a titer of 0.9 log $TCID_{50}$ per 0.2 ml. The DNA hybridization assay for viruria was negative. Interferon therapy was begun at 5×10^6 U subcutaneously twice a day. Because granulocyte counts were initially 1,500/mm^3 and decreased to 1,000/mm^3 on therapy, no increase in interferon dosage was attempted. Four-hour post-injection interferon levels on two occasions during therapy were 109 and 87 U/ml. Quantitative tissue culture of serial urine specimens showed an increase in the viral titer to 2.5 log $TCID_{50}$ per 0.2 ml. Eye examination 4 and 10 days after treatment began showed no progression of disease. After 3 weeks of therapy, interferon was stopped. Eye examination at 4 weeks revealed progression of the retinitis in the right eye; at a 3-month follow-up, the disease appeared to have stabilized with the formation of a retinal scar. At this time, the left eye was normal. The patient was not further seen at our institution; however, information was obtained 6 months later that a recrudescence of his CMV infection progressed to bilateral eye involvement, resulting in blindness. He died shortly thereafter from disseminated CMV infection.

Case 4. A 46-year-old male was hospitalized with Hodgkin's disease and decreased vision in the left eye. He had a history of rheumatoid arthritis. Ten months previously, he had received prednisone for generalized adenopathy and splenomegaly. A month later, an axillary lymph node biopsy revealed mixed cellularity Hodgkin's disease, and several cycles of combination chemotherapy followed. Three months before referral, a tonsillar lesion showed Hodgkin's disease; this was treated with radiation therapy. Prednisone was continued. Vision loss was also noted at this time. Serial funduscopic examination revealed progressive chorioretinitis characteristic of CMV, with bilateral involvement, but mainly in the left eye. Culture of a urine specimen showed 6.9 log $TCID_{50}$ per 0.2 ml, and the DNA hybridization assay was markedly positive. Prednisone therapy was rapidly tapered from 50 mg to 5 mg per day over a 2-week period; interferon was begun at 5×10^6 U twice daily in the middle of this interval. Ten days later, viruria as measured by quantitative culture and DNA hybridization was sharply decreased, and eye lesions appeared unchanged. Attempted escalation of the interferon dosage by 50% resulted in a granulocyte count of 700/mm^3, and the dose was therefore restored to the previous level. At this dosage, granulocyte counts stabilized at about 1,500/mm^3. Four-hour post-injection interferon levels ranged between 60 and 84 U/ml. Shortly after prednisone had been tapered, a hypotensive episode occurred which was treated with 1 g of methylprednisolone, followed by large, gradually tapering doses of oral prednisone. After this, viruria increased, and eye lesions worsened. Although interferon was stopped for 1 week after a 5-week course of therapy, it was decided to reinstitute therapy for 4 more weeks while steroid therapy was further

reduced. During and after the second course of therapy, there were two cycles of increased viruria. Eye lesions stabilized, and the disease was quiescent at 3- and 8-month follow-ups. Visual acuity was intact in the right eye but was reduced to perception of hand motion in the left. Viruria was still present at the last follow-up. Although no tissue culture quantitation was done, the intensity of the DNA hybridization signal suggested an approximate titer of 5 log $TCID_{50}$ per 0.2 ml.

MATERIALS AND METHODS

Viral isolation and assay. CMV was grown in tissue culture with human foreskin fibroblasts, and quantitative cultures and the DNA hybridization assay were performed as previously described (3). The latter assay provides an alternative method for quantitation of viruria in which results are obtained in 24 h, in contrast to the 3-week duration of quantitative tissue culture. The detection threshold of the hybridization assay is about 10^3 50% tissue culture infective doses ($TCID_{50}$) per ml. Fresh urine specimens from study patients were quantitatively cultured at least weekly, and samples of these specimens were frozen for immediate or subsequent hybridization assays.

Interferon therapy. Patients who underwent interferon therapy had characteristic eye lesions of CMV retinitis, consisting of opaque white or yellow lesions with various degrees of retinal hemorrhage and sheathing, as previously described (4). All had CMV viruria and antibody to CMV as measured by the standard complement fixation test or radioimmunoassay (11). Human leukocyte interferon was prepared from buffy coat leukocytes under the direction of K. Cantell of the Finnish Blood Transfusion Service. The partially purified preparation had a specific activity of 5×10^6 to 7×10^6 U/mg of protein. Doses of 5×10^6 to 18×10^6 U were administered intramuscularly or subcutaneously twice a day. Doses were escalated to tolerance as determined by leukopenia or systemic symptoms. The duration of therapy ranged from 3 to 5 weeks per course. This use of human leukocyte interferon was under a Food and Drug Administration investigational new drug permit and approved by the Stanford University Human Subjects Committee. Interferon levels in the blood were measured in a vesicular stomatitis virus plaque reduction assay as previously described (7).

Eye lesions were examined and photographed weekly; progression of the retinitis was determined by comparison of the areas of involvement from week to week.

RESULTS AND DISCUSSION

We observed progression of CMV retinitis in four severely immunosuppressed patients after treatment with maximally tolerated doses of buffy coat-derived interferon. Patients with AIDS appear to pose an especially difficult therapeutic problem in antimicrobial therapy because the mechanisms causing their profound and unremitting immunosuppression are not at present understood. In the patient without AIDS (case 4), administration of interferon was augmented by concomitant reduction of exogenous immunosuppressive therapy. This could have contributed to the eventual stabilization of his retinitis, whereas all the AIDS patients had progressive visual loss. Interferon dosage was limited by granulocytopenia in three of the patients we treated; one AIDS patient did not have granulocytopenia when given 36×10^6 U of interferon per day, but did have constitutional symptoms severe enough to be considered dose limiting.

Virological monitoring of the patients revealed that, consistent with past experience, interferon therapy did not result in eradication of viruria. In two patients in the present study, it is possible that the decreases in viruria seen during therapy could be related to interferon, analogous to observations previously reported in congenitally infected infants (1), but it is also apparent that this is not a consistent effect since the other two patients actually had increased viruria. In one instance we also demonstrated that viremia was not eradicated after 2 weeks of high-dose interferon treatment.

In the management of CMV retinitis, the first step is to reduce or eliminate exogenous immunosuppressive therapy. When this fails or when immunosuppression is due to disease, the threat to vision and the paucity of current therapeutic options will encourage further efforts at experimental antiviral therapy. Still to be evaluated are the various highly purified and recombinant DNA-derived interferons, which could have biological properties different from those of the buffy coat-derived material we used in this study. Newer nucleoside analogs with greater in vitro activity against CMV than the agents currently in clinical use should be available for experimental use in the near future. As before, close virological monitoring will be a necessary adjunct to clinical studies, since a virological response should precede any beneficial impact on clinical disease.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grant AI-05629 from the National Institutes of Health and in part by an unrestricted grant from Research to Prevent Blindness, Inc. S.C. received Bank of America-Giannini Fellowship awards in 1981 and 1982.

We thank Sue Rosno and Linda Roark for assistance with the study protocol.

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