Cyclosporine-induced deterioration in patients with AIDS

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Eight patients with AIDS (acquired immune deficiency syndrome) but free of life-threatening infection were treated with the immunosuppressive drug cyclosporine for a mean of 53.9 days. The serum cyclosporine levels were maintained in the desired therapeutic range. All eight patients experienced severe toxic symptoms, which necessitated discontinuation of cyclosporine therapy in six. The serum levels of creatinine, urea and potassium rose during treatment and fell after therapy was stopped. The total leukocyte count, hemoglobin level, platelet count, total T-cell count, and T4- and T8-cell counts all fell markedly during treatment. The total leukocyte count, platelet count, and T4- and T8-cell counts rose after therapy was stopped, but the hemoglobin level remained low. No patient experienced resolution of symptoms during therapy, and the condition of all patients improved after treatment was stopped. The results of this pilot study indicate that cyclosporine does not alleviate, and may worsen, the symptoms and laboratory findings in patients with AIDS.

On a recours à l'immunosuppression par la cyclosporine chez huit sidatiques sans infection présentant un risque vital. La durée moyenne du traitement est de 53,9 jours; on maintient la cyclosporinémie dans les limites thérapeutiques voulues. Il survient des symptomes toxiques graves chez chacun des huit malades, motivant l'arrêt du traitement chez six d'entre eux. La créatinine, l'urée et le potassium sériques s'élèvent durant le traitement et retombent après son arrêt. On observe aussi une chute brutale de l'hémoglobinémie, de la numération totale des leucocytes, des plaquettes, de celles des lymphocytes T, des T4 et des T8. Les numérations remontent après arrêt du traitement, mais non l'hémoglobinémie. Aucun malade ne voit ses symptômes s'amender pendant le traitement; l'état de chacun d'entre eux s'améliore après l'arrêt. Ce travail pilote donne à penser que la cyclosporine, loin d'améliorer les altérations cliniques et biologiques du SIDA, les aggrave plutôt.

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AIDS (acquired immune deficiency syndrome) is a uniformly fatal disorder caused by infection with HIV-1 (human immunodeficiency virus type 1).[1] The virus infects T4 lymphocytes and causes progressive destruction and loss of these cells as well as functional derangements in surviving lymphocyte populations (T8 cells and B cells).[2] Antiviral agents have not been as effective as one would hope, although some improvement in survival rates has been reported for patients treated with zidovudine (AZT).[3,4] Attempts at immune modulation or reconstitution by a variety of procedures have not been successful.[5-9]

Although the precise pathogenesis of HIV-1-associated disease is unknown, several investigators have suggested that in AIDS the immune system may play a role in its own destruction.[10,11] This may be a consequence of an autoimmune phase of disease progression in which various types of effector cells, sensitized by infected lymphocytes, participate in the destruction of both HIV-1-infected and healthy cells. In this sense the T4-cell deficiency in AIDS may be analogous to

other autoimmune cytopenias such as aplastic anemia.[12]

Accordingly, several investigators have speculated that some form of immunosuppressive therapy may help restore adequate levels of CD4(+) lymphocytes or prevent further diminution of the CD4(+) pool in HIV-1-infected people.[10,12] One compound that has been considered in this regard is cyclosporine. In addition to its effectiveness in preventing allograft rejection[13,14] this drug prevents lymphocyte activation and has produced significant improvement in other autoimmune cytopenias, such as aplastic anemia and pure red cell aplasia,[15] without severe toxicity. Indeed, reports from French investigators suggested that cyclosporine treatment in AIDS can lead to transient clinical and immunologic improvement.[16] While we were carrying out our study, work was progressing that suggested that cyclosporine may interfere with the binding of HIV-1 to receptor sites on susceptible cells.[17]

We carried out a pilot study of cyclosporine therapy in patients with AIDS whose condition was clinically stable to determine whether cyclosporine produced sustained improvement in immunologic variables, particularly circulating numbers of T4 cells.

Methods

The study was done in 1986 with patients who had been referred from teaching hospitals of the University of Toronto. Approval was obtained from the ethics committees of the institutions. All eligible patients had group IV disease (classification of the US Centers for Disease Control, Atlanta[3]) and included subjects with Kaposi's sarcoma who had not received chemotherapeutic agents for at least 1 month and subjects with *Pneumocystis carinii* pneumonia who had been treated for one or two episodes. All the patients were seropositive for HIV-1 and had given informed consent to participate in the study. Exclusion criteria included life-threatening, active opportunistic infection, involvement in other therapeutic trials, inability to adhere to the follow-up schedule or the presence of severe renal or hepatic dysfunction.

All the patients were followed weekly in the outpatient clinic at Toronto General Hospital with a variety of laboratory tests, including routine blood work, urinalysis and determination of serum levels of urea, creatinine, electrolytes, aspartate aminotransferase, alanine aminotransferase and bilirubin. Plasma cyclosporine levels were determined by radioimmunoassay (courtesy of Dr. Pui-Yuen Wong). Phenotypic analysis of B and T cells and T-cell subsets was done with a dual-laser Epic V fluorocytometer with fluorescein-isothiocyanate-conjugated anti-B1 (Coulter Electronics of Canada Ltd., Toronto) and OKT4, OKT8 and OKT11 (Ortho Diagnostics Inc., Mississauga, Ont.) monoclonal antibodies. Laboratory investigations were started 4 weeks before initiation of cyclosporine therapy and were scheduled to continue for 4 weeks after cessation of treatment. In addition, lymphocyte blastogenic responsiveness[18] to phytohemagglutinin (PHA), concanavalin A and pokeweed mitogen as well as leukocyte 2-5A synthetase levels[19,20] were measured twice weekly, and the patients were clinically evaluated two or three times a week.

Cyclosporine therapy was begun at a dosage of 7.5 mg/kg per day, divided into two doses, adjusted to maintain a 12-hour plasma trough level of 100 to 150 ng/ml (the usual dosage in transplantation and autoimmune disorders). We intended to continue therapy for 120 days. Cyclosporine treatment was to be stopped if opportunistic infections, serious renal toxic effects or unexpected complications believed to be due to cyclosporine developed.

Isolation of HIV-1 from the patients' peripheral blood lymphocytes was attempted by a coculture procedure 1 to 3 weeks before initiation of cyclosporine therapy, 3 to 6 weeks after initiation of treatment and 2 to 4 weeks after cessation of therapy. The lymphocytes were stimulated with PHA in RPMI-1640 medium supplemented with 10% fetal calf serum (Gibco Laboratories, Mississauga) and 2 days later were added to peripheral blood lymphocytes from healthy donors that had been stimulated with PHA 24 hours earlier. After 24 hours of coculture at 37°C at a concentration of 10^6 cells/ml (500 000 cells/ml each from the patient and the donor) the cells were washed in Hanks' balanced salt solution and resuspended to a concentration of 10^6/ml in RPMI-1640 medium supplemented with 10% fetal calf serum and exogenous, purified interleukin-2 (2% volume for volume) (Boehringer Mannheim [Canada] Ltd., Dorval, PQ). The cells were washed and reincubated with fresh peripheral blood lymphocytes from healthy donors prestimulated with PHA every 3 days for a total of 30 days or until the cultures became positive for detectable progeny HIV-1, as determined with the reverse transcriptase assay.[17]

Results

Of the nine patients (all men) entered into the study six presented with *P. carinii* pneumonia and three had Kaposi's sarcoma (Table I). In one patient (patient 9) massive intravascular hemolysis developed, which may have been due to cyclosporine therapy; he was withdrawn from the study after 13 days of treatment. Side effects are reported for this patient (Table I), but his laboratory data were incomplete and were therefore excluded from subsequent analyses. Therapeutic blood levels of cyclosporine were maintained in seven of the eight remaining patients, as indicated by a concomitant rise in serum levels of creatinine, urea and potassium. In patient 8 the cyclosporine dosage had to be decreased because of persistently elevated serum creatinine levels. All the patients achieved cyclosporine levels in the target range. Serum levels of

creatinine, urea and potassium returned to normal in all the patients after cyclosporine therapy was stopped.

A number of symptoms, including those usually associated with AIDS (e.g., nausea, vomiting, fatigue and malaise), developed or worsened during cyclosporine therapy (Table II). Eight of the patients experienced debilitating fatigue that severely curtailed their general activity compared with their activity before treatment. A mean weight loss of 2.4 kg occurred during therapy. These findings prompted early termination of cyclosporine treatment in six subjects (patients 1 to 6). Cessation of therapy resulted in partial resolution but not disappearance of the symptoms in all eight of the patients followed. No weight gain occurred after withdrawal of cyclosporine. Two of the patients with Kaposi's sarcoma experienced a marked increase in the size, swelling and pain of their lesions while receiving cyclosporine therapy. Progression of these symptoms stopped within 72 hours after withdrawal of the drug.

The new symptoms apparently induced by cyclosporine included anorexia and muscle pain. Interestingly, the side effects most commonly associated with the immunosuppressive use of cyclosporine (including tremor, gum hypertrophy, paresthesias and hypertension) occurred only rarely in our patients. Cessation of cyclosporine therapy resulted in alleviation of anorexia, muscle pain and twitches, headache, tremor, gum hypertrophy and paresthesias.

The hematologic and immunologic findings before, during and after cyclosporine therapy are summarized in Table III. The lymphocyte and platelet counts as well as the hemoglobin levels fell during treatment, whereas the granulocyte counts remained stable. Two subjects (patients 6 and 8) required several blood transfusions during the study, so the observed changes in their hemoglobin levels underestimate the severity of their anemia. After therapy was stopped the hemoglobin levels stabilized but did not rise, whereas increases were observed in the total leukocyte and platelet counts. Treatment with cyclosporine apparently resulted in a sharp decrease in the total numbers of T cells (i.e., both T4 and T8 lymphocytes). After cessation of therapy a substantial increase in the total number of lymphocytes but not T4 cells was observed. No changes in the lymphocyte blastogenesis profile or in the levels of 2-5A synthetase were noted during or after therapy.

HIV-1 was isolated from only one of four patients tested before cyclosporine treatment was started (Table IV). In contrast, the virus was isolated from all six patients tested during therapy. Cultures from five of the six patients also yielded the virus after treatment was stopped.

Because of cyclosporine's toxicity for this population no patient completed the intended 120 days of therapy. In six cases the patient asked to withdraw from the study, and in the other two the decision to stop therapy was made by the attending physician. An important consideration in each case was the obvious lack of benefit and the potential harmful effects of continuing therapy.

Compliance was generally good, although one

Table II — Adverse effects of cyclosporine therapy

Symptom	No. of patients (n = 9)
Nausea	8
Vomiting	4
Fatigue	8
Malaise	7
Anorexia	6
Muscle pain	6
Muscle twitches	2
Headache	2
Tremor	2
Gum hypertropy	2
Paresthesias	1
Hypertension	0

Table I — Characteristics of patients with AIDS (acquired immune deficiency syndrome) who received cyclosporine therapy

Patient no.	Age, yr	Presenting diagnosis	Time since diagnosis of AIDS, mo	Duration of cyclosporine therapy, d	Mean daily cyclosporine dosage, mg/kg	Mean serum cyclosporine level, ng/ml	Mean serum creatinine level during treatment (and standard deviation [SD]), μmol/L
1	35	*Pneumocystis carinii* pneumonia	15	23	6.9	150	176 (26)
2	40	Kaposi's sarcoma	11	46	7.2	118	172 (31)
3	37	Kaposi's sarcoma	14	65	6.9	177	108 (5)
4	26	*P. carinii* pneumonia	6	42	6.3	177	121 (5)
5	29	*P. carinii* pneumonia	10	50	6.3	142	131 (7)
6	42	*P. carinii* pneumonia	12	49	4.6	196	139 (18)
7	38	*P. carinii* pneumonia	5	86	7.0	66	119 (12)
8	30	*P. carinii* pneumonia	6	70	6.9	89	154 (10)
9	42	Kaposi's sarcoma	20	13	6.9	70	96 (5)
Mean (and SD)	35 (6)		11 (5)	49 (22)	6.6 (0.8)	131 (48)	135 (26)

subject (patient 7) had to be reminded to keep his appointments.

Discussion

We found that cyclosporine treatment in patients with AIDS was not beneficial and that such therapy was demonstrably toxic in a high proportion of cases. Although target plasma levels of cyclosporine were achieved in seven of eight patients, no patient experienced resolution of any major symptom or improvement in any laboratory variable. The condition of most of our patients deteriorated during cyclosporine therapy.

Most patients who receive cyclosporine for transplantation or treatment of autoimmune disease tolerate the drug well for long periods despite its nephrotoxicity.[21,22] In our study certain side effects of cyclosporine (pain, muscle twitching, fatigue and anorexia) were frequent and severe; this finding suggests that cyclosporine may be unusually toxic for patients with AIDS. The condition of all the patients became subjectively worse while they were receiving cyclosporine. In contrast, their clinical status generally improved after therapy was stopped. The occurrence of unexpected, severe, debilitating symptoms and hematologic side effects during cyclosporine therapy is consistent with the high rate of toxic effects seen with other drugs in patients with advanced HIV-1-associated disease.[23-25]

Our patients experienced increased lymphopenia and further diminution of the T4-cell population during treatment with cyclosporine. The blastogenic response to a variety of T-cell and B-cell mitogens remained unchanged both during

Table IV — Isolation of HIV-1*

Patient no.	Reverse transcriptase level in culture fluids, cpm/ml†		
	Before treatment	During treatment	After treatment
1	ND	ND	ND
2	931	863 521	1 629 657
3	ND	407 688	735 214
4	1 362 521	842 693	803 795
5	1 238	741 391	1 734
6	1 079	984 618	756 286
7	ND	ND	ND
8	ND	635 220	543 794

*Isolation of HIV-1 from peripheral blood lymphocytes was attempted 1 to 3 weeks before initiation of cyclosporine therapy, 3 to 6 weeks after initiation of treatment and 2 to 4 weeks after cessation of therapy.
†ND = not determined.

Table III — Mean values of hematologic and immunologic variables before, during and after cyclosporine therapy

Variable (and normal range)	Mean result (and SD)		
	Before treatment*	During treatment†	After treatment‡
Leukocyte count (4.0–11.0 × 10^9/L)	3.854 (1.242)	3.149 (1.254)]	4.432 (0.932)
Lymphocyte count (> 1.0 × 10^9/L)	1.272 (0.516)	0.884 (0.597)	1.181 (0.709)
Granulocyte count (> 1.5 × 10^9/L)	1.785 (0.612)	1.782 (0.318)	2.440 (0.231)
Hemoglobin level (140–160 g/L)	127 (14.3)	111 (15.1)	106 (18.0)
Platelet count (150–400 × 10^9/L)	198 (57.5)	155 (52.9)	240 (63.6)
Total no. of T cells (> 0.90 × 10^9/L)	0.888 (0.420)	0.584 (0.452)	0.872 (0.598)
Total no. of T4 cells (> 0.63 × 10^9/L)	0.118 (0.159)	0.073 (0.111)	0.110 (0.162)
Total no. of T8 cells (> 0.30 × 10^9/L)	0.378 (0.167)	0.238 (0.177)	0.436 (0.278)
2-5A synthetase level (< 30 units)	176 (73)	234 (147)	234 (105)
Lymphocyte blastogenic response			
Pokeweed mitogen (> 10 000 cpm)	8 147 (2 897)	9 284 (4 105)	9 632 (5 043)
Phytohemagglutinin (> 50 000 cpm)	63 332 (27 523)	66 919 (30 109)	51 557 (31 083)
Concanavalin A (> 50 000 cpm)	22 899 (12 019)	26 233 (18 788)	15 423 (7 287)

*Mean of two values before therapy.
†Mean of last four values during therapy.
‡Mean of three values after therapy.

and after therapy. It has been reported that 2-5A synthetase levels are persistently elevated in patients with AIDS;[20] in our study, cyclosporine therapy did not appreciably affect this variable.

Cyclosporine is generally considered to be nonmyelotoxic,[26] but it has been reported to produce anemia in patients with juvenile-onset diabetes mellitus.[21] In our study the platelet counts, total leukocyte counts, T4- and T8-lymphocyte counts and hemoglobin levels decreased during therapy and, except for the hemoglobin levels, rose toward pretreatment values after therapy was stopped. These hematologic effects may reflect a direct effect of cyclosporine on the bone marrow in patients with AIDS. Alternatively, they may represent the effect of increased replication of HIV-1 under conditions of cyclosporine therapy as a consequence of possible suppression of effective anti-HIV-1 immune responsiveness. Indeed, we found that HIV-1 could routinely be isolated more efficiently from our patients' peripheral blood lymphocytes after cyclosporine therapy was started than before therapy. This may reflect the inhibitory effects of the drug on effective cell-mediated anti-HIV immunity in our patients. Such immune responsiveness is thought to play a key role in limiting viral replication in the infected host.

Previous results obtained in vitro showed that cyclosporine may impede the binding of HIV-1 to its CD4 receptor and hence prevent viral internalization and replication.[17] The drug can also inhibit interleukin-2-dependent T-cell proliferation, an essential step in HIV-1 replication.[26] Our findings indicate the difficulty of extrapolating from results obtained with tissue culture to the clinical situation.

Our results may also have implications for HIV-1-seropositive patients who undergo organ transplantation and are treated with cyclosporine. Such patients clearly will have to be monitored for the development of more advanced HIV-1-associated disease leading to AIDS. Our findings suggest that one possible reason for such potential deterioration is enhanced replication of HIV-1 in patients treated with cyclosporine.

Our results indicate that cyclosporine should not be used to treat patients with AIDS, in spite of an appropriate rationale for the use of this drug and supportive in-vitro observations.

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