

A Phase II Study of Effect of Addition of Trichosanthin to Zidovudine in Patients with HIV Disease and Failing Antiretroviral Agents

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ABSTRACT

Patients infected with HIV, including those with AIDS-related complex and AIDS, and failing treatment with antiretroviral agents such as zidovudine, have been evaluated following addition of trichosanthin to the antiretroviral agent regimen. This ribosomal inhibitory protein is specifically cytotoxic for HIV-infected macrophages and lymphocytes. Ninety-three patients were treated with trichosanthin, using a schedule of weekly, then monthly, intravenous injections of 1.2 mg of drug in combination with antiretroviral agents, usually zidovudine. Side effects included myalgias, fevers, mild elevation in liver function tests, and mild-moderate anaphylactic reactions, which respond well to therapy with steroids and/or benedryl. Reversible mental status changes were noted in two patients, both receiving concomitant therapy with ddI.

Clinical responses to trichosanthin treatment were monitored primarily by changes in laboratory parameters, particularly levels of CD4⁺ T lymphocytes. In the total population evaluated for efficacy (85 patients) there was a significant increase in CD4⁺ cell levels after initiation of trichosanthin therapy. A second analysis performed on 72 patients measured the rate of change of CD4⁺ cells during therapy, using an "area under the curve" analysis. During therapy there was a median increase of 1.2 cells/mm³/month. In patients in the top 25th percentile, this increase was greater than 8.4 cells/mm³/month. In 59 of the 72 patients, responses could also be monitored by comparing the rate of loss of CD4⁺ cell levels on antiretroviral agents (zidovudine or ddI) alone, during the year prior to initiation of trichosanthin, to the rate of change when trichosanthin was added to the treatment regimen. During the period before trichosanthin treatment (311 ± 11.7 days) the median loss of CD4⁺ cells was 6.91 cells/mm³/month. Addition of trichosanthin to the treatment regimen resulted in a median gain of 1.1 CD4⁺ cells/mm³/month.

INTRODUCTION

HUMAN IMMUNODEFICIENCY VIRUS (HIV) infection causes chronic progressive depletion of CD4⁺ lymphocytes (CD4⁺ cells) and infection of macrophages resulting in acquired immune deficiency (AIDS). Currently zidovudine, an analog of thymidine, is the primary antiviral drug used in the treatment of HIV infection,^{1,2} although other agents with a similar mechanism of action, such as dideoxyinosine (ddI), have been evaluated in clinical trials.³⁻⁵ These agents are effective in inhibiting

viral replication, and can stabilize the CD4⁺ cell levels, but chronically infected cells, especially those of the mononuclear phagocytic cell lineage, may represent an intrinsic limitation to the clinical efficacy of nucleoside analogs such as zidovudine.⁶ Severe toxicity, particularly involving the bone marrow, is associated with higher doses of zidovudine treatment and the beneficial effects of the drug in AIDS patients diminish after prolonged therapy⁷; HIV strains resistant to zidovudine have been observed in treated patients.⁸ These findings have prompted the search for alternative drugs for the treatment of

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HIV infection, particularly agents with a different mechanism of action.⁹

Trichosanthin, derived from the root tuber of *Trichosanthes kirilowii*, belongs to a class of proteins including ricin,¹⁰ momorcharins,¹¹ and trichokirin,¹² which inhibit ribosome synthesis and are cytotoxic for a range of mammalian cells. Pharmaceutical-grade trichosanthin has been used for many years in China as an abortifacient, and as an anticancer agent for choriocarcinoma.¹³ Trichosanthin inhibits HIV replication in cultured T lymphoblastoid cells and is specifically cytotoxic for infected monocyte/macrophages.¹⁴ These properties have led to its evaluation for the treatment of HIV-infected patients.^{15,16}

Two phase I dose escalation studies using this agent in HIV-infected patients have been reported. In the first, trichosanthin was obtained from China, and 51 patients with advanced HIV disease were removed from all other drugs and given three weekly injections of trichosanthin at doses ranging from 10 to 30 $\mu\text{g/kg}$.¹⁵ Dose-related side effects were similar to those seen with other members of this class of compounds, and included reversible hypoalbuminemia. The maximal tolerated dose was 30 $\mu\text{g/kg}$ per dose, and was defined by severe reversible myalgia. A non-dose-related side effect was mental status change presenting as reversible dementia, which could progress to coma. This was associated with CD4^+ cell levels less than $50/\text{mm}^3$. The second study utilized trichosanthin manufactured in the United States (GLQ 223), and involved the treatment of 18 patients at doses of 5–36 $\mu\text{g/kg}$ as a single intravenous injection.¹⁶ Side effects were similar between the two studies, and no evidence of bone marrow toxicity was noted, suggesting that combination therapy with zidovudine and trichosanthin would be well tolerated. In those patients with CD4^+ cell levels over $50/\text{mm}^3$, who received three injections of drug, a significant increase in CD4^+ cell levels was noted.¹⁵

In the study reported here, 93 patients were selected who had been receiving a stable dose of antiretroviral agents for up to 3 years prior to initiation of study, and who had failed these drugs, as manifest by decreases in CD4^+ cell levels. These patients remained on the same dose of zidovudine (or in few cases ddI), and received trichosanthin (1.2 mg/dose) weekly, then monthly. The response to trichosanthin treatment was primarily evaluated by changes in CD4^+ lymphocyte levels, using three different methods of analysis. In 85 patients, CD4^+ cell levels were available at periods of time before and after trichosanthin treatment, and these were compared with the rate of change in CD4^+ cell levels. In 72 patients at least 2 CD4^+ cell levels following trichosanthin treatment were available, allowing additional evaluation by rate of change of CD4^+ cell levels, as analyzed by the area under the curve formed by the CD4^+ cell levels during trichosanthin therapy. In 59 patients CD4^+ cell levels were also available during the year prior to trichosanthin therapy as well as after therapy. This permitted comparison of the rate of CD4^+ cell changes before and after therapy. The results indicate there was a significant loss in CD4^+ cell level prior to initiation of trichosanthin, and significant gain in the levels after its initiation.

SUBJECTS AND METHODS

Trichosanthin characteristics

Clinical-grade trichosanthin was obtained from the Shanghai Institute of Organic Chemistry, provided as a crystalline product

dissolved in sterile distilled water, in ampoules containing 1 ml of solution (1.2 mg/ml). The five lots used in the clinical studies were tested by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing, which demonstrated a pure protein product of characteristic molecular weight and isoelectric point.¹⁵ For clinical use, the appropriate amount of trichosanthin was diluted into 500 ml of 5% dextrose in sterile water just prior to administration.

Clinical protocol

This was a single-institution treatment protocol, performed on an outpatient basis, with the clinical protocol approved by the Project Inform Investigational Review Board. All patients gave informed consent. Patients were between the ages of 18 and 65 years, diagnosed with HIV disease by positive serology, who had been receiving either zidovudine or ddI for at least 1 year prior to therapy, and who had failed these drugs. This was manifest either by failure to stabilize CD4^+ values, with CD4^+ cell levels decreasing by 50 cells/ mm^3 /year, or by significant decreases in CD4^+ cell levels on two sequential measurements just prior to therapy. Trichosanthin was administered intravenously (i.v.) at a dose of one ampoule per week, once a week for 2 weeks, then monthly, infused over a period of 2 hr. The exception to this was the first group of 18 patients, who received 0.4–0.6 mg as the first dose, then full dose thereafter (inpatient dose escalation). In all patients, before the first infusion, two test doses of 0.5 and 5 μg in 1 ml of 5% dextrose in water were administered as an intravenous bolus, with observation for 30 min between each test dose. All patients were intended to receive at least three infusions and to have CD4^+ cell levels measured at least twice in addition to the baseline evaluation, but maximum number of infusions were not specified. Trichosanthin would be expected to be synergistic with antiretroviral agents, because, unlike antiretroviral agents,⁶ it is able to inhibit HIV replication in chronically infected macrophages.¹⁴ Therefore these agents were continued during treatment, and patients remained on the same dose of zidovudine or ddI during the study period. All patients with CD4^+ cell levels less than $200/\text{mm}^3$ were receiving aerosolized pentamidine (300 mg each month); some patients with CD4^+ cells between 200 and $300/\text{mm}^3$ were also receiving this therapy. Those patients who had an episode of herpes simplex were receiving acyclovir (600 mg/day), and remained on this therapy through the study period. Some patients were receiving transfer factor, prepared as previously described, and administered intramuscularly every 2 weeks prior to study, as prophylaxis against viral infections,¹⁷ and some patients were receiving dextran sulfate (1800 mg/day) during the prestudy period. In all cases both treatments were discontinued at least 4 months prior to start of study.

To enter the study, patients must have had peripheral blood CD4^+ cell levels between 50 and $500/\text{mm}^3$, and had to be positive for HIV infection by serology. They must have had granulocyte levels greater than $1000/\text{mm}^3$, platelet levels greater than $100,000/\text{mm}^3$, a hemoglobin level greater than 8.5 g/ml, with bilirubin less than 1.5 times normal, aspartate lysine transaminase (SGOT) and aspartate serine transaminase (SGPT) less than three times normal, and creatinine less than 1.5 mg/dl. Pregnant or breast-feeding women or those not practicing birth control were excluded from entry.

Prior to entering the study patients signed an informed

consent, and history and physical examination, Karnofsky score, 12-lead electrocardiogram (EKG), and chest X-ray were performed. Additionally, nuclear magnetic resonance imaging or computerized tomography of the brain, or neuropsychiatric testing, were required. Laboratory tests included a complete blood count, urinalysis, serum chemistry panel, erythrocyte sedimentation rate, β_2 -microglobulin, p24 antigen and antibody levels, all within 2 weeks of the first trichosanthin administration. Physical examination was repeated every 2–4 weeks, and laboratory values at least once every 2 months.

Trichosanthin was administered in an outpatient setting. Physicians were in attendance during its administration, with vital signs recorded every 30 min during infusion and for 1 hr thereafter. Patients were then discharged into the care of a medically trained person, or informed layman, who performed mental status tests every 4 hr for the next 60 hr. Those patients with suspected mental status change occurring during this time were given dexamethasone (8 mg every 4 hr) until symptoms resolved, usually in the hospital. Physical examinations of all patients were repeated by physicians 48 hr after infusion.

When allergic reactions occurred during therapy, patients were treated with benedryl (25-mg i.v. bolus) and dexamethasone (8 mg i.v.) and the infusion was continued after resolution of all symptoms (usually 15 min) with no further problems; for subsequent infusions, such patients were premedicated with oral benedryl and dexamethasone the night before infusion, then every 4–8 hr until the morning after the next infusion.

Serum HIV p24

HIV p24 antigen was determined centrally using a Coulter (Hialeah, FL) p24 antigen capture enzyme-linked immunosorbent assay (ELISA).^{15,18} Serum p24 antigen is captured by anti-p24 monoclonal antibody and detected by labeled polyclonal anti-p24 antibody. The detection limit of the assay is 7–10 pg/ml and test-to-test variation is 15%.

Serum anti-p24 antibody

Serum anti-p24 antibody was determined as previously described¹⁸ by binding to recombinant p24 protein coupled to microbeads. Antibody binding was then detected by reaction with fluorescently labeled anti-human antibody and expressed as fluorescence units.

β_2 -microglobulin

The serum protein β_2 -microglobulin was determined by an ELISA using anti- β_2 -microglobulin as capture antibody (Abbott Laboratories, Abbott Park, IL).

Lymphocyte analysis

Peripheral blood was reacted with fluorochrome-labeled monoclonal antibodies Leu2, -3, or -4, (Becton Dickinson, Mountain View, CA), and following lysis of erythrocytes the leukocytes were analyzed by two-color flow cytometry within 24 hr of collection.¹⁵

Patient populations

Ninety-three patients were analyzed for safety, meeting the criteria of receiving at least one infusion of trichosanthin, and

being evaluable for 7 days afterward. Patients were followed for at least 7 days after the last infusion, and any new abnormalities on physical examination, or elevations greater than threefold on laboratory parameters from baseline and above normal range, during the 7-day period were recorded.

Patients were evaluated for efficacy by determining their overall gain in CD4⁺ cells after initiation of trichosanthin. Eighty-five patients met the criteria for this evaluation, having at least one CD4⁺ cell level determination in addition to baseline. In 72 patients at least two CD4⁺ cell level determinations in addition to baseline values were available, permitting analysis of the rate of change in CD4⁺ cell levels using the “area under the curve” determination. Fifty-nine patients had CD4⁺ cell levels evaluated for at least 1 year prior to beginning of therapy with trichosanthin, while receiving zidovudine alone. It was possible to calculate the change in cell levels prior to therapy in these patients, which allowed comparison of the pretreatment change with the posttreatment change. The CD4⁺ cell levels at initiation of trichosanthin treatment in the above subgroups were comparable, ranging from 230 ± 115 cells/mm³ to 236 ± 125 cells/mm³ (Table 1).

Calculation of area under curve

To obtain the rate of change of CD4⁺ cell level after initiation of trichosanthin therapy, serial determinations were used, beginning either from day 0 or from the most proximal CD4⁺ determination prior to start of therapy (which had to be no greater than 90 days prior to the first infusion) and continued to the final available determination. After calculating the area under curve formed by these determinations, baseline was subtracted, and the area was then divided by the period of observation (days) to give a mean CD4⁺ cell level per day (cells/mm³/day). The same method was used to calculate the change in CD4⁺ cell levels during the year prior to trichosanthin therapy.¹⁹

Statistical analysis

Statistical tests were performed using Statistical Package for Social Sciences (SPSS, Inc., Chicago, IL). Analysis of posttrichosanthin changes in CD4⁺ cell levels indicated a nonnormal distribution (Kolmogorov–Smirnov test). For this reason, median and quartile values were used to analyze this distribution, rather than a mean and standard deviation. Similarly, for the 59 patients for whom pretrichosanthin rates of change of CD4⁺ cell level were available, the differences between pre- and posttreatment CD4⁺ rates of change were not normally distributed. A nonparametric analysis (Wilcoxon signed rank sum test) was therefore used to assess the statistical significance of this comparison.

RESULTS

Demographics

The majority of the patients were male homosexual Caucasians (Table 1). Most were asymptomatic although 17 had prior illnesses defining them as having AIDS-related complex (ARC), and 11 had AIDS. Patients who exited the study after receiving fewer than three doses of trichosanthin did so for reasons other than toxicity. The database was frozen August 20, 1990; at that

TABLE 1. DEMOGRAPHICS OF PATIENTS TREATED WITH TRICHOSANTHIN

	Total population	Patients assessed for efficacy by:		
		Pre- and post-CD4 ⁺ cell level	Posttrichosanthin CD4 ⁺ cell level	Pre- and posttreatment CD4 ⁺ cell level
Number	93	85	72	59
Male	91	83	70	58
Caucasian	91	83	70	57
Mean age (SD)	37 (6.85)	37 (6.65)	37 (6.76)	37 (6.63)
Mean weight (SD)	74.89 (10)	74.5 (10)	75 (10)	74.2 (8)
Clinical status				
Asymptomatic	65	60	52	45
ARC	17	15	13	9
Hairy leukoplakia	5	5	5	4
Herpes zoster	5	4	3	1
Thrush	4	3	2	1
Chronic hepatitis B	1	1	1	1
Molluscum contagiosum	1	1	1	1
Lymphadenopathy	1	1	1	1
AIDS	11	10	7	5
AIDS dementia	1	1	1	1
<i>Pneumocystis pneumonia</i>	1	1	1	1
Kaposi's sarcoma	9	8	5	3
Mean CD4 cell level at start of trichosanthin therapy:	230 (115)	233 (116)	230 (119)	236 (125)

time all patients had had their last infusion within 90 days. The mean CD4⁺ levels at the start of trichosanthin therapy were 230 ± 115 cells/mm³. Most patients had CNS evaluation by MRI or CT prior to entering the study, and all were negative, except for two patients who had a past history of AIDS-related dementia and showed atrophy on MRI, but no evidence of HIV encephalopathy by MRI or clinical examination. There was no overall difference between the demographics of the total number of patients treated, and evaluated for safety (93 patients), and the subgroups evaluated for change in CD4 cell levels after trichosanthin therapy (Table 1). The 34 patients who withdrew from therapy after only 1 infusion did so for nonmedical reasons.

Trichosanthin treatment

Ninety-three patients were treated on this protocol. Eighty-seven were failing zidovudine (AZT) at doses of 300–500 mg/day and remained on the same dose of this drug during therapy; 6 were failing ddI at a dose of 250 mg twice daily and also remained on this drug during trichosanthin therapy, except for the two patients suffering from drug-related toxicity during trichosanthin therapy who were then switched to zidovudine. All were treated with at least one dose of trichosanthin, and received follow-up care for at least 7 days; these patients are included in the safety analysis. Most patients received a full ampoule (1.2 mg) of trichosanthin for each dose, but 18 patients received an inpatient dose escalation, usually consisting of 0.4–0.6 mg on the first dose, 0.8 mg on the second dose, and a full 1.2 mg for the doses thereafter. These were the first patients entered onto the protocol.

Concomitant medications

Seventy-one patients received 4–6 infusions of trichosanthin (mean, 4.9 ± 1.9); 22 received 7–10 infusions. In addition to the

antiretroviral agents, AZT and ddI, which all patients continued to receive, 49 patients were receiving acyclovir because of previous episodes of herpes simplex; all but 1 remained on this therapy during trichosanthin infusions. Forty-four patients were receiving aerosolized pentamidine monthly as prophylaxis against *Pneumocystis pneumonia*; these patients also continued this treatment during trichosanthin therapy.

Side effects

The 93 patients evaluated for side effects of trichosanthin (Table 2) were evaluated for any laboratory or clinical abnormalities during the weekly or monthly infusions or within 7 days after the last infusion. All patients developed myalgias and arthralgias primarily of the upper trunk, hands, and arms, with myalgia extending up into the neck. This was moderate in intensity, appearing within 36 hr of infusion and lasting for 2–3 days. It was treated with nonsteroidal antiinflammatory agents; hospitalization was never required. All patients developed fever 36–60 hr after the first infusion. These were also mild to moderate in intensity, the highest level being 103°F. Both the fevers and myalgias tended to decrease by the time of the third and subsequent infusions. The principal laboratory changes during therapy were in the liver function tests. Thirteen patients had abnormalities in liver function tests; 6–7 of these patients had entered the study with abnormally elevated values. This increase occurred a mean of 68 days after the first infusion after an average of three infusions, and returned to baseline in 47 to 51 days even though the trichosanthin infusions were continued for an average of two additional infusions. In one additional patient there was an elevation of the total serum bilirubin level, which also returned to baseline during subsequent trichosanthin infusions. This patient was documented to have developed hepatitis

TABLE 2. SIDE EFFECTS ASSOCIATED WITH TRICHOSANTHIN TREATMENT

Laboratory test	N	Normal range	Value at baseline	Value at peak/nadir	Days between first infusion and peak value	Days to return to baseline
SGOT (g/dl)	10	3–45	47 ± 6	281 ± 104	68 ± 37.5	47 ± 26
SGPT (g/dl)	13	3–50	62 ± 18	242 ± 117	68 ± 37.5	51 ± 30
Alkaline phosphatase (g/dl)	1	30–128	61	645	31	30
Total bilirubin (g/dl)	1	0.1–1.5	0.6	5.9	12	31
LDH (g/dl)	5	90–225	172 ± 41	597	68 ± 37.5	43 ± 16
Hemoglobin ^a (g/dl)	4	12–15	11.8 ± 2	8.2 ± 0.4	98.5 ± 37.5	62 ± 2
Platelets ^b (×1000/cm ³)	2	140–440	162 ± 9	94 ± 7	129	
Clinical						
Anaphylactoid reactions	20	N/A	N/A	N/A	67 ± 30	N/A ^c
Mental status change	2 ^d	N/A	N/A	N/A	3–4 months	24 hr
Myalgia	93	N/A	N/A	N/A	0–36 hr	7 days

^aDecreased below 10 g%.^bDecreased below 90,000/mm³.^cNo patient developed a second anaphylactoid reaction on retreatment. N/A, Not applicable.^dBoth patients were receiving ddI.

A while on therapy. Two and 4 patients experienced a mild reversible thrombocytopenia and anemia, respectively, and 20 patients developed non-life-threatening anaphylactoid reactions during therapy. This reaction typically developed after the fourth infusion, and presented as erythroderma, conjunctival edema, and occasional upper airway wheezing without abnormalities in the lower airway. In most cases benedryl allowed completion of the infusion and these patients were pretreated with dexamethasone prior to the subsequent infusions; all tolerated it well.

Mental status changes were seen in two of the six patients receiving ddI as their concomitant antiviral agent. One of these patients developed a mild syncopal episode during the first infusion, and had three subsequent infusions without problems. Another developed a grand mal seizure 36 hr after the first infusion. He received six subsequent infusions without problems. Because seizures are a known side effect of ddI, the patients were removed from ddI and restarted on zidovudine prior to giving them additional infusions.

Three deaths occurred in the group of patients reported here. One patient had Kaposi's sarcoma, and had received multiple transfusions because of zidovudine toxicity, resulting in hemolytic anemia. This death, of hemolytic anemia, occurred 50 days after the last infusion. A second patient died of *Pneumo-*

cystis pneumonia 187 days after the last infusion, and a third died of bronchogenic carcinoma 180 days after the last infusion, which on retrospective analysis of the chest X-ray taken at study entry was present at start of study.

Changes in CD4⁺ cell levels

Comparing CD4⁺ cell levels at initiation of trichosanthin treatment in 85 of the 93 patients with maximum values posttreatment demonstrated an increase in the median CD4⁺ cell levels (230 and 262, respectively, $p < 0.001$ by Wilcoxon signed rank sum test). To define more clearly the rate of change of CD4⁺ cell levels, 72 patients were analyzed using the area under the curve procedure (AUC; Table 3). Patients were gaining CD4⁺ cells after trichosanthin treatment (median gain, 1.19 cells/mm³/month); the top 25% were gaining more than 8.37 cells/mm³/month. The response to trichosanthin treatment is also illustrated by analysis of 59 patients in whom it was possible to determine the rate of change of CD4⁺ cell level before and after treatment, using the AUC method (Table 3). The distribution of posttrichosanthin therapy change in CD4⁺ cells in this subgroup is similar to that in the parent group of 72 patients; this similarity is also reflected by the quartile values for the group and subgroup (Table 4).

TABLE 3. INFLUENCE OF TRICHOSANTHIN TREATMENT ON CD4⁺ LYMPHOCYTE LEVEL

Patient population	Number of patients	Change in CD4 ⁺ cells/mm ³ /month		
		25th percentile	Median	75th percentile
Evaluation after trichosanthin only	72	−5.60	+1.19	+8.37
Evaluation before and after trichosanthin				
Before	59	−12.51	−6.91	−0.24
After	59	−4.44	+1.10	−8.34

TABLE 4. PRETREATMENT LABORATORY PARAMETERS RELATED TO RATE OF CHANGE OF CD4⁺ CELL LEVEL AFTER TRICHOSANTHIN TREATMENT (n = 80)

Pretreatment parameter	Number of patients	Means of parameter within quartiles according to rate of gain of CD4 ⁺ cells after trichosanthin treatment				p value (analysis of variance)	
		Lowest 25%	25–50%	50–75%	Top 25%	Between groups	Linearity
CD4 ⁺ count ^a	72	252	233	195	236	0.534	0.504
CD8 ⁺ count ^a	72	960	860	833	700	0.252	0.050
p24 antigen (log) ^a	63	0.66	0.051	0.28	0.00	0.093	0.013
p24 antibody (log) ^a	57	1.76	2.01	2.20	3.01	0.096	0.020

^aBaseline determination (most proximal value to initiation of therapy).

In these 59 patients, during the monitoring period before trichosanthin therapy (197 ± 84 days), the median change in CD4⁺ cell level was a loss of 6.91 cells/mm³/month, and only 14 of the 59 patients had a stabilization of CD4⁺ cell level in the pretrichosanthin-monitoring period. It should be noted that these 14 patients met the second of the entry criteria, having a decreasing CD4⁺ cell level on two successive evaluations prior to trichosanthin treatment. Analysis of the distribution of the rates of change of CD4⁺ cell level prior to trichosanthin treatment (Table 3) shows that 75% of the patients were losing CD4⁺ cells faster than 0.24 cells/mm³/month, with a median rate of change of -6.91 cells/mm³/month. In comparison, after trichosanthin was added to the treatment regimen there was a median gain of 1.10 cells/mm³/month, with 25% of the patients gaining CD4⁺ cells faster than 8.34 cells/mm³/month. Twenty patients received dexamethasone after 1 of the infusions because of anaphylactoid reactions, and were pretreated with dexamethasone prior to subsequent infusions; 15 of them are included in the 59 evaluated for efficacy. There is no indication that the response of these patients differed from those not receiving dexamethasone. Eight of the 15 had a net gain of CD4⁺ cells and 7 had a net loss.

A paired comparison of the rates of change of CD4⁺ cells before and after trichosanthin treatment in this group of 59 patients was tested for statistical significance by the nonparametric Wilcoxon signed rank sum test, as the differences between pre- and posttherapy rates were not normally distributed. The difference in rate of loss of CD4⁺ cells before and after trichosanthin treatment was highly significant ($p < 0.0001$). In the top 25%, this represented a gain of more than 103 CD4⁺ cells/mm³/year. Overall, 47 of the 59 patients showed a decreased rate of loss of CD4⁺ cells after trichosanthin treatment.

Other laboratory parameters

The rate of change in CD4⁺ cell level after initiation of trichosanthin treatment was compared with other laboratory parameters at study entry. These included baseline CD4⁺ and CD8⁺ cell levels, p24 antigen and antibody, sedimentation rate, and β_2 -microglobulin. Thus the patients were divided into quartiles on the basis of their posttrichosanthin change in CD4⁺ cell level and the mean value for the baseline laboratory measurements in these subgroups determined. The statistical

significance of these distributions was evaluated by an analysis of variance. The results are summarized in Table 4.

There was no relationship between either the rate of change in CD4⁺ cell level in the observation period before trichosanthin therapy or the level of these cells at the start of therapy, to the rate of change in CD4⁺ cell level after trichosanthin therapy. This last finding is important, as it shows that patients losing CD4⁺ cells at different rates before treatment respond equally well to trichosanthin.

There was a positive correlation between low p24 antigen levels and high p24 antibody levels at the start of therapy with response to trichosanthin. There was no significant relationship between pretreatment levels of β_2 -microglobulin or the erythrocyte sedimentation rate (ESR) and subsequent response to trichosanthin.

Changes in laboratory or clinical parameters

There was no significant change in other laboratory parameters after trichosanthin therapy. There was little change in disease-related status in any of the 93 patients treated. All patients who were symptomatic at study entry remained that way at conclusion of study, and all patients who entered the study with an AIDS diagnosis remained in that category. However, of the 17 patients with a diagnosis of ARC at start of trichosanthin therapy, 2 converted to AIDS during the therapy with trichosanthin. One of these developed MAI, and a second developed *Pneumocystis pneumonia*.

DISCUSSION

In this phase II study, 93 patients who had persistent decreases in CD4⁺ cell level while being treated with zidovudine or ddI had monthly infusions of trichosanthin added to their regimen. Response to this combined therapy was determined by assessment of the changes in CD4⁺ cell levels, or rate of change of CD4⁺ cell level during and after therapy. The change in CD4⁺ cell level was assessed primarily by determining the rate of change using the area under the curve parameter.¹⁹ Among the 72 patients in whom the rate of change in CD4⁺ cell level could be assessed after initiation of trichosanthin there was a median increase of 1.2 cells/mm³/month, and the 75th percentile patients gained 8.53 cells/mm³/month (Table 3). Because these

patients were failing antiretroviral therapy prior to trichosanthin this is encouraging, but the interpretation of such changes is difficult in an uncontrolled study. Therefore the changes in CD4⁺ cell levels among 59 patients during or after therapy was compared to the changes in this parameter during the year just prior to addition of trichosanthin to the antiretroviral regime (Table 3). On antiretroviral drugs alone, patients were losing CD4⁺ cells at a rate of 6.9 cells/mm³/month; the addition of trichosanthin allowed this trend to reverse, with a gain of 1.1 cells/mm³/month. It is notable that posttreatment CD4⁺ values and the rates of change of CD4⁺ values after therapy have a distinctly nonnormal right-skewed distribution. In contrast, pretreatment baseline CD4⁺ values and the rate of loss of CD4⁺ cells before treatment have a symmetrical normal distribution. This implies that there may be heterogeneity in the response to trichosanthin.

Grouping patients into quartiles on the basis of response to trichosanthin allows assessment of the laboratory values on entry that are predictive of best response. There was little difference in the entry CD4⁺ values, but those patients showing best response to trichosanthin were those with the lowest level of p24 antigen and highest levels of p24 antibody, both of which are correlated with good prognosis.^{20,21} Discriminate analysis was used to analyze these pretreatment parameters in an attempt to identify patients responding best to trichosanthin. Although classification into good and bad responders was much better by this analysis than by chance, the relationship of response to the pretreatment parameters measured so far proved not to be strong enough to predict the putative subpopulation responding best to trichosanthin (Robins Byers and Baldwin, unpublished observations, 1993).

The dose of the drug—1.2 mg/patient—was predicted to be the highest dose that would be tolerated in conjunction with ddI and AZT. The principal toxicity of zidovudine is bone marrow suppression. Pancreatitis and mental status deterioration are associated with ddI. Trichosanthin has been associated with myalgia, allergic reactions, and mental status change.^{15,16} In this combined study, all patients developed myalgias, and about a quarter noted erythroderma and/or periorbital edema, usually after the third or fourth infusion. None of these caused withdrawal from the study; pretreatment with acetaminophen and benedryl prior to subsequent infusions, or with dexamethasone when reactions occurred during therapy, allowed uneventful continuation of infusions. Two of six patients receiving ddI developed reversible mental status changes, necessitating discontinuation of ddI during trichosanthin therapy. Overall the combination produced no new or exaggerated side effects, and higher doses should be tried.

Trichosanthin acts to suppress HIV replication in acutely infected T lymphocytes,¹⁴ but its clinical value resides in its effect on macrophages. A 3-hr exposure to trichosanthin will inhibit viral replication in macrophages for 5 days, and produce cell death 2–3 weeks later. Infection in these cells is noncytopathic, and they can continue to produce virus for long periods of time, thus serving as a substantial reservoir for HIV *in vitro*. The dose used here—about 240 ng/ml of serum in a 70-kg patient with 5 liters of blood—should provide substantial killing, because the ID₅₀ (50% infective dose) in the *in vitro* studies is 50 ng/ml.¹⁴ Although the pharmacokinetics of trichosanthin are rapid (2–4 hr) the dose regimen should provide the 3-hr exposure necessary to prevent reactivation for 5 days, and cause death of infected cells 2–3 weeks later.

It was initially surprising that the main effect was an increase in CD4⁺ cell levels, and not on the p24 antigen levels. If, however, the principal target of trichosanthin was the infected macrophages, this would be the expected outcome. Chronically HIV-infected macrophages release a potent interleukin 1 (IL-1) inhibitor that blocks proliferation of T cells by interfering with production of IL-2.²² Inhibition of viral replication, and killing of these cells, should release the block and allow normal T cell proliferation. Because of the low number of patients entered into the study with elevated p24 antigen levels, an effect on this parameter would have to be large to be statistically significant. Subsequent studies using larger numbers of such patients may demonstrate clearing of viremia. Also, if the p24 antigen levels are the result of synthesis by another cell population, such as T lymphocytes, higher doses may be required to show viral clearing.

CD4⁺ cell levels have been considered acceptable as an indicator of response to drugs or disease progression.^{23–25} Their limitations have been highlighted by two similarly designed studies, the results of one suggesting that they are not a relevant prognostic indicator,²⁶ the other indicating they are.²⁷ An assessment of the status of cellular immunity requires information on both the number and the function of the cells, and the conflicting data in the latter two studies may be partially due to the fact that CD4⁺ cell levels provide only part of the picture. This limitation must also apply to this study. However, the marked increase in the cell levels during trichosanthin therapy in patients who had failed antiretroviral agents indicates that larger studies with higher doses can be expected to clear viremia and possibly prolong survival.

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REFERENCES

1. Volberding PA, Lagakos SW, Kock MA, *et al.*: Zidovudine in asymptomatic human immunodeficiency virus infection. *N Engl J Med* 1990;322:941–949.
2. Fischl MA, Richman DD, Hansen N, *et al.*: The safety and efficacy of zidovudine (AZT) in the treatment of subjects with mildly symptomatic human immunodeficiency virus type 1 (HIV) infection. *Ann Intern Med* 1990;112:727–737.
3. Lambert JS, Seidlin M, Reichman RC, *et al.*: 2',3'-Dideoxyinosine (ddI) in patients with the acquired immunodeficiency syndrome or AIDS-related complex. *N Engl J Med* 1990;322:1333–1340.
4. Cooley TP, Kunches LM, Saunders CA, *et al.*: Once-daily administration of 2',3'-dideoxyinosine (ddI) in patients with the acquired immunodeficiency syndrome or AIDS-related complex. *N Engl J Med* 1990;322:1340–1345.

5. Yarchoan R, Plunda JM, Thomas RV, *et al.*: Long-term toxicity/activity profile of 2',3'-dideoxyinosine in AIDS or AIDS-related complex. *Lancet* 1990;ii:526-528.
6. Perno CF, Yarchoan R, Cooney DA, *et al.*: Inhibition of human immunodeficiency virus (HIV-1/HTLV-III_{Ba-L}) replication in fresh and cultured human peripheral blood monocytes/macrophages by azidothymidine and related 2',3'-dideoxynucleosides. *J Exp Med* 1988;168:1111-1125.
7. Richman DD, Fischl MA, Grieco MH, *et al.*: The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex: A double blind placebo-controlled trial. *N Engl J Med* 1987;317:192-197.
8. Larder BA, Darby G, and Richman DD: HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. *Science* 1989;243:1731-1734.
9. Merigan TC: Issues in the development of more effective antiviral therapies for HIV infection. In: *Current Issues in the Management of Patients with HIV Infection*. Krown SE and Borden EC (eds.). Mediscript, London, 1991, pp. 3-19.
10. Endo Y and Tsurugi K: RNA N-glycosidase activity of ricin A chain. Mechanism of action of the toxic lectin ricin on eukaryotic ribosomes. *J Biol Chem* 1987;262:8128-8130.
11. Yeung HW, Li WW, Geng Z, *et al.*: Trichosanthin, α -momorcharin and β -momorcharin: Identity of abortifacient and ribosome-inactivating proteins. *Int J Peptide Protein Res* 1988;31:265-268.
12. Casellas P, Dussosoy D, Falasca AI, *et al.*: Trichokirin, a ribosome-inactivating protein from the seeds of *Trichosanthes kirilowii* Maximowicz. Purification, partial characterisation and use for preparation of immunotoxins. 1988;176:581-588.
13. Lau IF, Saksena SK, and Chang MC: Further studies on the trichosanthin-induced termination of pregnancy. *Contraception* 1980;21:77-86.
14. McGrath MS, Hwang KM, Caldwell SE, *et al.*: GLQ223: An inhibitor of human immunodeficiency virus replication in acutely and chronically infected cell of lymphocyte and mononuclear lineage. *Proc Natl Acad Sci USA* 1989;86:2844-2848.
15. Byers VS, Levin AS, Waites LA, *et al.*: A phase I/II study of trichosanthin treatment of HIV disease. *AIDS* 1990;4:1189-1196.
16. Kahn JO, Kaplan LD, Bambertoglio JG, *et al.*: The safety and pharmacokinetics of GLQ223 in subjects with AIDS and AIDS related complex: A phase I study. *AIDS* 1990;4:1197-1204.
17. Steele RW, Myers MG, and Vincent MM: Transfer factor for the prevention of varicella zoster infection in childhood leukemia. *N Engl J Med* 1980;303:355-359.
18. Jacobson MA, Bacchetti P, Kolokathis A, *et al.*: Surrogate markers for survival in patients with AIDS and AIDS-related complex treated with zidovudine. *Br Med J* 1991;302:73-78.
19. Rowland M and Tozer TN: *Clinical Pharmacokinetics. Concepts and Applications*. Lea & Febiger, Philadelphia, 1989, pp. 459-461.
20. Sheppard HW, Ascher M, Lange W, *et al.*: Immune response, immune activation. *J AIDS* 1991;4:704-712.
21. Allain JP, Laurian MD, Paul DA, *et al.*: Long-term evaluation of HIV antigen and antibodies to p24 and gp41 in patients with hemophilia. *N Engl J Med* 1987;317:1114-1121.
22. Locksley RM, Crowe S, Sadick MD, *et al.*: Release of interleukin 1 inhibitory activity (Contra-IL1) by human monocyte-derived macrophages infected with human immunodeficiency virus in vitro and in vivo. *J Clin Invest* 1988;82:2097-2105.
23. de Wolf F, Lange JMA, Houweling JTM, *et al.*: Numbers of CD4+ cells and the levels of core antigens of and antibodies to the human immunodeficiency virus as predictors of AIDS among seropositive homosexual men. *J Infect Dis* 1988;158:615-622.
24. Stein DS, Korvick JA, and Vermund SH: CD4+ lymphocyte cell enumeration for prediction of clinical course of human immunodeficiency virus disease: A review. *J Infect Dis* 1992;165:352-363.
25. Phillips AN, Lee CA, Elford J, *et al.*: Serial CD4 lymphocyte counts and development of AIDS. *Lancet* 1991;i:389-392.
26. Aboulker J-P and Swart AM: Preliminary analysis of the Concorde Trial. *Lancet* 1993;341:889-890.
27. Cooper DA, Gatell JM, Kroon S, *et al.*: Zidovudine in persons with asymptomatic HIV infection and CD+ counts greater than 400 per cubic millimeter. *N Engl J Med* 1993;329:297-303.

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2. Siu-Hong Chan, Fanny S.-J. Hung, Denize S.-B. Chan, Pang-Chui Shaw. 2001. Trichosanthin interacts with acidic ribosomal proteins P0 and P1 and mitotic checkpoint protein MAD2B. *European Journal of Biochemistry* **268**:7, 2107-2112. [[CrossRef](#)]
3. Pang-Chui Shaw, Sandrine Mulot, Sun-Kai Ma, Qiong-Fang Xu, Hong-Bing Yao, Shen Wu, Xiao-Hui Lu, Yi-Cheng Dong. 1997. Structure/Function Relationship Study of Tyr14 and Arg22 in Trichosanthin, a Ribosome-Inactivating Protein. *European Journal of Biochemistry* **245**:2, 423-427. [[CrossRef](#)]