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TITLE: Prospective Double-Blind Study of Zidovudine (AZT) in Early Stage HIV Infection

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) A prospective, double-blind study of treatment of early HIV infection with low-dose Zidovudine (3'-Azidothymidine) will answer questions of cost/toxicity versus clinical benefit in this important infection. A dosage of 200mg taken orally every six hours was calculated to optimize treatment benefits and minimize toxic side-effects. In addition to routine urinalysis, chemistries and hematologies, comprehensive history and physical data will be taken including: viral culture, HIV p24 antigen, helper/suppressor cell numbers, cytomegalovirus and toxoplasmosis IgG levels, Beta-2-microglobulin levels, and a variety of psychological tests. Over 200 military and civilian patients will be studied for a minimum of two years.					
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FOREWORD

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INTRODUCTION

High cost and toxicity of the anti-viral drug 3'-Azido-thymidine (Zidovudine) makes necessary a careful study of its possible benefits. Chronic low-level dosing of a population with signs and symptoms of early stage HIV infection may sufficiently inhibit viral replication to alter the rate of disease progression, with acceptable toxicity. Data provided by the present study will help answer these cost/benefit questions. This study will also provide valuable experience in application of state of the art HIV culture and antigen detection methods as well as CD4 cell counts, Toxoplasma and Cytomegalovirus antibody tests, to an investigation of clinical AIDS. This study is a valuable adjunct to clinical care, providing necessary improvements to clinical standards for compulsory follow-up asymptomatic HIV infected patients at FAMC. ()

BACKGROUND

Zidovudine inhibits HIV replication in vitro with little toxic effect on uninfected lymphocytes (5). However, hematopoietic progenitor cells are more sensitive to Zidovudine toxicity. Bone marrow suppression with neutropenia, macrocytosis, and anemia requiring blood transfusions occurred in 21% of 282 patients receiving 1500mg, orally, per day (6). Retrovir prolonged the life of these patients, reduced the number of opportunistic infections, and partially reversed skin test

anergy in 30% while increasing the number of CD4 cells. After 12 weeks, the CD4 cell numbers declined to pre-treatment values in HIV infected patients with <200 CD4 cells/ml at start but remained elevated through week 20 in patients with >200 CD4 cells/ml (1). More Zidovudine recipients (17/33) cultured negative for HIV than placebo recipients (5/19). A trend to decreasing serum p24 antigen levels was seen in Zidovudine recipients.

This milestone study (1) supports the need to critically examine CD4/CD8 cell numbers, serum p24 antigen levels, and results of culture for HIV (as well as the total clinical assessment) as indicators of potentially positive responses to treatment.

Published rates of HIV isolation by tissue culture range from 41% (7/17) in clinically healthy homosexual/bisexual men to 80% (4/5) in that group with lymphomas (9). HIV was isolated from 27/35 (77%) of African AIDS patients and 5/9 (55%) of African ARC patients (10). These isolations were performed using co-cultivation of patient lymphocytes with mitogen stimulated, normal human peripheral blood lymphocytes. While this appears to be the optimal method to recover free HIV, adequate recovery of HIV by co-cultivation of infected patient lymphocytes with CEM cultures is possible (11). The use of cell lines is far less labor intensive and makes the present study feasible.

HIV p24 antigenemia appears to be bi-phasic and inversely related to p24 antibody levels (12). Published rates of p24 antigen positivity range from 4% (13) to 35% (14) in clinically well, seropositives. An increasing percentage of p24 antigen

positivity was reported in the progression of HIV seropositives from persistent generalized lymphadenopathy (25%) to ARC (56%) to AIDS (70%), showing that p24 antigenemia is related to disease progression (13). Retrovir in relatively high doses (1200 and 1500mg, but not 600mg per day) reduced p24 antigenemia in studies involving 9 and 16 treated patients respectively (15, 16).

The gradual depletion of CD4 lymphocytes after HIV infection coincides with a spectrum of clinical disease ranging from asymptomatic infection to a profound state of immunodeficiency (7). Using the Walter Reed staging scale to indicate rate of clinical progression; 50% of the FAMC seropositive population progressed one WR stage or more in mean follow-up of 16.3 months (8). This rate, combined with studies of the rate of change of other laboratory tests such as the CD4 cell number, is sufficient to allow a two-year prospective double-blind placebo controlled study of Zidovudine with 100 patients in each arm. Other details of study background, including Zidovudine pharmacokinetics, phase I safety and tolerance evaluations are described in detail in the original protocol proposal.

Measurement of antibody to Cytomegalovirus (CMV) is another important aspect of the present protocol. Human CMV activates HIV replication in tissue culture, and vice versa (17). Additional data consistent with CMV co-activation of HIV includes: the high prevalence of CMV infection in homosexual men and patients with AIDS; frequent CMV reinfections in homosexual men; documented immunosuppressive effects of CMV infection; and

epidemiologic evidence for CMV involvement in Kaposi's sarcoma (18). A high titer of CMV antibody was independently associated with subsequent AIDS in a cohort of 1835 homosexual men seropositive for HIV (19). Evidence against CMV co-activation was reported on 50 homosexual men (38% seropositive) indicating that those developing AIDS symptoms did not differ from healthy seropositives in the ability to isolate CMV (20). Measurement of CMV antibodies in the present study will clarify the co-factor issue, as well as measure the patients ability to continue to produce antibody to a common viral co-infection of HIV seropositives.

Measurement of Toxoplasma (Toxo) antibodies is critical in the present study. Incidence of Toxo encephalitis in AIDS patients ranges from 3-80% in various U.S. hospitals, with an overall risk of 6-12% in AIDS patients with positive toxo serological findings (21, 22). Serological tests are useful in screening for toxo-induced disease.

Serum beta 2-microglobulin (B2M) levels were found elevated in a homosexual man who presented with peripheral generalized lymphadenopathy and later developed AIDS (23). This is a non-specific response, and elevated serum B2M levels can result from renal failure, autoimmune disease, infectious disease, and tumors (24). However, serum B2M levels are reported to parallel disease activity in AIDS (24). Serum B2M levels are easily and accurately measured by commercial ELISA kits. This assay may be useful in following HIV disease progression and therapy.

OBJECTIVES/PURPOSE

The primary objective is conduct a prospective, double-blind study to define the safety and efficacy of chronic, low-dose Zidovudine in early HIV infection. Use of the Walter Reed staging scale permits clinical classification of HIV disease stages and allows a more precise determination of the relationship of this staging scale to clinical prognosis. Psychological testing and other laboratory findings, such as p24 antigenemia levels, will better define stages of disease progression.

METHODS OF APPROACH

Study design is not changed from the original protocol. About 300 patients from Denver General Hospital (DGH) and an equal number from routine clinical lab testing (SMA-18 chemistry, hepatitis and syphilis serologies, hematologies, pregnancy, chest X-ray, skin test, urinalysis, quantitative immunoglobulin tests) and comprehensive physical and psychological examinations; this involved over 1200 CMV and Toxo antibody tests, over 1200 CD4/CD8 determinations, 600 HIV ELISA and Western blot tests, 255 quantitative HIV p24 antigen tests, 200 beta 2-microglobulin tests, and 200 lymphocyte co-cultures.

Over 70 DGH and 30 FAMC patients were entered into study. This lower rate of patient accrual results from a revision of US Army Regulation 40-501 which places many potential military enrollees in a TDRL status with geographic mobility. Also, more strict entry criteria were imposed at a late stage of study

design. The revised criteria limited patients to those maintaining 200-500 CD4 (helper) cells on entry, since these patients would most quickly show any positive drug effects.

B2M, Toxo IgG, CMV IgG, and HIV p24 antigen levels are measured with the following commercial kits: Toxo and CMV antibodies--a fluorescent immunoassay (25); B2M by ELISA (24); and HIV p24 antigen is measured with an Abbott kit (15). Kits are used according to package insert instructions.

Helper (CD4) and supressor (CD8) lymphocytes are counted on a Coulter model C flow cytometer, using two-color (green= FITC, red= Phycoerythrin) fluorochrome analysis as described below. Diurnal variations are minimized by collecting whole blood for complete blood count and lymphocyte immunophenotyping during the same venipuncture between 0800 and 1100 hours. The absolute lymphocyte count is done within four hours of blood collection into an EDTA tube; phenotyping is done with 24 hours of blood collection. The coulter immunolyse procedure is used to eliminate red cells. Lymphocytes are labeled with commercial monoclonal antibodies (Coulter or Becton-Dickinson) in accordance with manufacturers recommendations. Appropriate controls for non-specific binding and accurate definition of lymphocyte subpopulations in addition to checks of optical and electronic alignment are built into sample processing procedures. The laboratory participates in a commercially contracted quality assurance program (FAST systems Inc.).

HIV infected peripheral blood lymphocytes are detected by co-cultivation of Ficoll separated patient lymphocytes with CEM cells (ATCC TIB 195). CEM cells are an established human T-cell line, free of HLA Dr antigen, and interleukin-2 independent. They were successfully used by Montagnier and others to isolate HIV from patients at risk for AIDS (26, 27). The method involves maintaining CEM cells in a humidified (37 C, 5% CO₂) incubator, in media of RPMI 1640 containing 20% fetal bovine serum, Penicillin (250 units/ml), Streptomycin (250 ug/ml), Amphotericin B (2 Ug/ml), and L-Glutamine (300ug/ml)--Medium A. About eight hundred thousand Ficoll separated patient lymphocytes from 10ml of whole blood are mixed one-to-one with CEM cells in Medium A containing interleukin-2 (Cellular Products, 10% final concentration) and Polybrene (Sigma, 2 ug/ml final concentration)--Medium B. After 6-7 days of incubation, supernatant fluids are assayed for HIV p24 antigen. At this time cultures are also fed with one hundred thousand CEM cells in Medium B. Following this feeding, culture supernatants are assayed every 3-4 days (to a total of 26-28 days) for HIV antigens. Tissue culture work is done according to CDC biosafety level 3 standards. A culture is scored positive if 50pg of HIV antigen or more is detected in the supernatant fluid.

CONCLUSIONS/RESULTS

Prevalence of CMV serum antibody is much higher than Toxo antibody in both Denver General Hospital (DGH) and FAMC patients. These results are shown in Table I.

TABLE I

	DGH	FAMC
	+/Total (%)	+/Total (%)
Toxo IgG	25/140 (18)	66/306 (22)
CMV IgG	50/50 (100)	175/199 (88)

Identification of Toxo seropositives was useful in screening candidates for chemotherapy.

A published upper limit of normal for B2M for the age range of most patients in the present study (17-36 yrs) is 1.77mg/L; while the limit for all males (n=58) was 2.31mg/L (28). Given these limits, the majority of study subjects sera fell over the upper limit. More data is needed before valid observations can be made on effects of AZT treatment.

Out of 68 patients on study for 2-40 weeks, 33 (48%) maintained their entry CD4 cell count; 21 (31%) have decreased by 50 cells or more; and 14 (20%) have increased. Large increases in CD4 numbers are not apparent, however small increases or maintenance of pre-treatment levels may be clinically important.

Out of 14 patients with measurable HIV serum antigen, 5 increased their serum levels by 50pg/ml or more, 7 decreased, and 2 remain the same. Further data will provide a valid answer to the question of treatment effects.

Out of 71 patients co-cultured with CEM cells, 23 (32%) produced HIV antigens in supernatant fluids. Nine of thirteen cultures (69%) that were done on patients with HIV antigenemia were positive. These isolation rates are consistent with recently published data of others (29).

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