Pentoxifylline Improves Cell-Mediated Immunity and Reduces Human Immunodeficiency Virus (HIV) Plasma Viremia in Asymptomatic HIV-Seropositive Persons

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The effects of pentoxifylline on immunologic and virologic parameters were evaluated in 10 human immunodeficiency virus–infected patients not receiving antiretroviral treatment. Patients were asymptomatic, had 300–500 CD4 cells/μL, and received pentoxifylline (1200 mg/day orally) for 4 months. Peripheral blood mononuclear cells were tested before and at five time points during therapy. A transient increase in CD4 cells was observed in 8 of 9 patients, and CD8 cells increased in 7 of 9 patients. These increases were negatively correlated with susceptibility to in vitro mitogen-stimulated apoptotic cell death. Pentoxifylline had a temporary effect on mitogen-stimulated cytokine production; thus, interferon-γ, interleukin (IL)-2, tumor necrosis factor-α, and lymphotoxin increased more than IL-10. Pentoxifylline also potentiated antigen-stimulated IL-2 production and proliferation in 8 of 9 patients and induced significant but transient decreases in plasma viremia in 7 of 9 patients. These preliminary findings suggest that pentoxifylline in vivo has an interesting but temporary influence on both immunologic and virologic parameters.

Pentoxifylline is a synthetic methylxanthine used in the therapy of peripheral vascular diseases. Pentoxifylline reduces tumor necrosis factor (TNF)-α production by murine and human antigen-presenting cells [1] by interfering with RNA accumulation [2]. In addition, pentoxifylline reduces TNF-α production, TNF-α serum concentration, and TNF-α–specific mRNA by peripheral blood mononuclear cells (PBMC) of human immunodeficiency virus (HIV)–seropositive persons [3, 4]. These effects may be secondary to a reduced activation of NF-κB and the subsequent modulation of long terminal repeat–controlled HIV replication [5]. A recent study indicated that pentoxifylline down-regulates TNF-α production of in vitro HIV-infected PBMC and influences the generation of type 2 more than of type 1 cytokines; the interferon (IFN)-γ–to–interleukin (IL)-10 ratio increased because of a more pronounced reduction in IL-10 than in IFN-γ production [6]. Thus, the action of
pentoxifylline is more complex than simply TNF-α–mediated impairment of HIV replication: pentoxifylline can directly influence the production of different cytokines.

Because pentoxifylline can modulate multiple immunologic and virologic factors, we analyzed the effects of in vivo pentoxifylline treatment of asymptomatic HIV-seropositive persons not receiving concomitant antiretroviral treatment.

Materials and Methods

Patients. Ten HIV-infected, antiretroviral-naïve asymptomatic patients with 300–500 CD4 cells/μL were selected for study. No patients received antiretroviral therapy in association with pentoxifylline. Patients were treated for 4 months with pentoxifylline (Trental; Hoechst Roussel Pharmaceuticals, Frankfurt, Germany; 1200 mg orally/day). All patients completed the study except number 5, who moved to a foreign country and was lost to follow-up.

Processing of samples. Whole blood was drawn in heparinized Vacutainer tubes (Becton Dickinson, Rutherford, NJ). PBMC were separated on lymphocyte separation medium (Organon Teknika, Durham, NC) and washed in PBS, and the number of viable leukocytes was determined by trypan blue exclusion and hemocytometry. PBMC were tested as fresh samples. EDTA-plasma samples, kept at 4°C, were processed within 2 h. After two-step centrifugation, the plasma samples were aliquoted, frozen, and kept at −80°C until use.

Apoptotic cell death (ACD). ACD was measured using a cellular DNA fragmentation ELISA kit (Boehringer Mannheim, Cologne, Germany) to detect bromo-deoxyuridine (BrdU)-labeled DNA fragments in the cytoplasm of PBMC. Peripheral blood lymphocytes (5 × 10^6/μL) were incubated for 18 h at 37°C in RPMI 1640 plus BrdU (1% final concentration) in T-75 flasks (Costar, Cambridge, MA). PBMC were subsequently washed, plated (5 × 10^5/μL) onto 96-well microtiter plates, and either stimulated (3 h) or not stimulated with pokeweed mitogens plus staphylococcal enterotoxin B (Sigma, St. Louis). Lysis was induced with a lysis solution, and the BrdU-labeled DNA fragments released in the culture supernatants were quantified by ELISA (first step: double helix DNA fragment; second step: BrdU). Data are indicated in relative optical density (OD; OD × 100).

In vitro cytokine production. Production of IFN-γ, IL-2, IL-10, TNF-α, and lymphotoxin (LT) by PBMC was determined by culturing PBMC in 24-well plates (Costar; 3 × 10^5/well) at 37°C in a moist, 7% CO_2 atmosphere. PBMC were either unstimulated or stimulated with phytohemagglutinin diluted 1:100. Cultures were supplemented with 5% pooled AB-positive human serum (Sigma), and supernatants were harvested and assayed after 48 h. Cytokine production was evaluated with the following ELISAs: IL-2, IFN-γ, IL-10, and TNF-α (all Picnta assays; Genzyme, Cambridge, MA) and TNF-β (Bender MedSystem, Vienna). Values for all cytokines were calculated from a standard curve of the corresponding recombinant human cytokine.

In vitro assays for T helper function. PBMC were unstimulated or stimulated with tetanus toxoid (Massachusetts Department of Health, Boston; 1:400 final concentration), irradiated allogeneic PBMC (50 Gy) from HIV-seronegative donors (ALLO), or synthetic HIV-1 peptides (Env, 2.5 mM final concentration; previously described [7]). For IL-2 production, 3 × 10^5 PBMC were cultured for 7 days in 96-well, flat-bottom plates (Costar) with anti–IL-2 receptor antibody anti-Tac (2 μg/mL). For proliferation, the cultures were pulsed on day 5 and harvested 20 h later. IL-2 production was determined by testing supernatants for the ability to stimulate the proliferation of an IL-2–dependent mouse lymphocyte line (CTLL). Four 2-fold dilutions in triplicate were plated with 8 × 10^4 CTLL/well in 96-well microtiter plates. After 24 h, the CTLL cultures were pulsed, and they were harvested 18 h later.

Quantitation of HIV-1 RNA by a reverse transcription (RT) polymerase chain reaction (PCR) assay. HIV-1 copy numbers were determined by a quantitative PCR assay (Ambicor HIV monitor; Roche Diagnostic Systems, Basel, Switzerland) based on a single, combined RT and amplification of a conserved region of the gag gene (a 142-bp sequence defined by primers SK462 and SK431) and on an internal quantitation standard (QS; a synthetic RNA molecule identical to the target except for a 20-bp-long probe binding region) [8]. HIV-1 RNA, isolated from 200 μL of plasma, was co-extracted with a known amount of QS and processed with a single round of RT and amplification using the enzyme rTth DNA polymerase [8]. HIV RNA copies/mL were determined using the following formula: OD (HIV) × dilution factor/OD (QS) × dilution factor × input copy number (QS) × 40 = HIV RNA copies/mL (the factor 40 had to be used to yield copies per milliliter since RNA was recovered from 1/8 or 25 μL of the original plasma volume). Differences between each time point were considered significant if (log of first determination − log of second determination)/0.15 was ≥1.96 (Colucci G; Roche Diagnostic Systems, Basel, personal communication).

Statistical analysis. Molecular data were analyzed using Friedman and Wilcoxon tests for repeated measures, using an SPSS (Chicago) statistical package.

Results

Pentoxifylline was well tolerated. No adverse effects were observed with pentoxifylline. Reduction in weakness was reported by 2 (22%) of 9 subjects. One patient who had been affected by therapy-resistant severe itching for 1 year reported the disappearance of this symptom upon pentoxifylline treatment.

Pentoxifylline modifies CD4 and CD8 cell counts and susceptibility to ACD. Pentoxifylline marginally increased CD4 cell counts in all but patient 1. The effect was transitory and evident sometime between weeks 4 and 12 after initiation of treatment. The median CD4 cell count was also improved when the pretherapy CD4 cell count was compared with that at weeks 4–12 (median, 83 cells/μL; range, −6 to 127). CD4 cell counts at the end of the study were unmodified compared with pretherapy counts (median, 46 cells/μL; range, −90 to 100) (figure 1A).

CD8 cell counts were similarly improved sometime between weeks 4 and 12 in 7 of 9 patients (median, 270 cells/μL; range, 33–359), and they were still elevated at week 16 in the 7 patients (median, 186 cells/μL; range, 68–642) (data not shown).
Figure 1. A, CD4 cell counts and mitogen-stimulated apoptotic cell death (ACD) (relative optical density = OD × 100) in peripheral blood lymphocytes. B, Mitogen-stimulated cytokine production by peripheral blood lymphocytes. A and B, HIV-seropositive patients before (T0) and during (T2, T4, T8, T12, T16) therapy with pentoxifylline. Production of phytohemagglutinin (PHA)-stimulated interleukin (IL)-2, lymphotoxin (LT), IL-10, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ is shown.
There was a mean increase of 20 (18%) CD4 cells and 24 (47%) CD8 cells above baseline counts at weeks 4–12 in the patients who improved. PBMC susceptibility to in vitro mitogen-stimulated ACD was reduced in 7 of 9 patients (patients 3 and 4 were exceptions) at week 16 compared with the pretreatment value. Pentoxifylline reduced in vitro susceptibility to ACD within 2 weeks in all patients, with the exception of patient 4. In all but 2 patients (1 and 4), susceptibility to ACD was maximally reduced concomitant with the greatest positive changes in CD4 and CD8 cell counts (weeks 4–12; figure 1A).

Pentoxifylline modifies mitogen-stimulated cytokine production. Phytohemagglutinin-stimulated production of IL-2, LT, TNF-α, IFN-γ, and IL-10 is shown in figure 1B. Pentoxifylline modified the production of all cytokines concomitantly and increased the production of IL-2, IFN-γ, LT, and TNF-α more than that of IL-10: IFN-γ production increased 2- to 3-fold in 6 of 9 patients (2, 6, and 7 were the exceptions); IL-2 production increased >3-fold in 4 of 9 patients (1, 4, 8, and 10); LT production increased >3-fold in 5 of 9 patients (1, 3, 4, 8, and 10); TNF-α production increased >3-fold in 6 of 9 patients (1, 3, 4, 6, 8, and 10); and IL-10 production increased >3-fold in 2 of 9 patients (3 and 4). The maximum boost in cytokine production was observed 2–12 weeks after initiation of therapy; this effect was self-limiting and vanished by week 16 in all but 3 patients (2, 7, and 8). Pentoxifylline-induced changes in cytokine production were related to positive variations in CD4 cell counts and reduced susceptibility of PBMC to ACD. One patient (no. 9), who had influenza infection at the time of blood collection, showed a drastic reduction in the production of all cytokines at week 12.

Pentoxifylline modifies antigen-stimulated IL-2 production and proliferation. PBMC of all patients were stimulated in vitro with tetanus toxoid, ALLO, or Env, and IL-2 production and proliferation were measured (figure 2A). For simplicity, we show only one point (1:4) of the IL-2 titration curve. An increase >5-fold over the basal value for at least two antigens was considered to be a positive increase. Antigen-stimulated IL-2 production by PBMC of 6 of 9 patients was defective before pentoxifylline. Pentoxifylline increased antigen-stimulated IL-2 production in 3 patients (1, 7, and 10) who did not show defects at time 0 and in 5 of 6 patients (2, 3, 6, 8, and 9) with defective T helper function before therapy, the only exception being patient 4 (only ALLO-stimulated IL-2 production increased during therapy).

Tetanus toxoid–stimulated IL-2 production increased in 8 of 9 patients (4 was the exception; average increase = 11, 9-fold above the basal value). Env-stimulated IL-2 production increased in 5 of 9 patients (1, 2, 7, 8, and 9; average increase = 5, 4-fold above the basal value). ALLO-stimulated IL-2 production increased in all patients (average increase = 17, 4-fold above the basal value). The effect, which was evident in most patients after 2 weeks of treatment, vanished by week 16. Similar results were observed when antigen-stimulated proliferation was analyzed (data not shown). Only 1 patient (no. 7) exhibited a decline in antigen (ALLO)-stimulated IL-2 production below entry levels after an initial increase.

Pentoxifylline modifies plasma viremia. Pentoxifylline-induced modifications in HIV-1 genome copies are shown in figure 2B. All patients but 1 (no. 4) had low to intermediate levels of HIV-1 genomic RNA before therapy (median = 14,452 copies/mL; range = 3120–11,7647). Pentoxifylline reduced HIV-1 genome copies in all but 2 patients (nos. 2 and 8; P = .0301, Friedman test). The HIV-1 genomic RNA median in the group was reduced to 8886 copies/mL at week 8. The reduction in virus load was significant at all time points (P < .05, Wilcoxon test for repeated measures), with the exception of week 16, relative to the baseline value. Plasma viremia reduction was significant compared with the pretherapy level in 3 of 7 subjects (7, 9, and 10) by week 2, in a fourth patient (no. 4) by week 4, and in 3 more patients (1, 3, and 6) by week 8. The effect of pentoxifylline was time-limited, as plasma viremia levels returned to pretreatment values by week 16 in 5 of 7 patients (1, 3, 6, 7, and 9).

Discussion

We evaluated the action of pentoxifylline on immunologic and virologic parameters, analyzing the effects of in vivo pentoxifylline in 10 antiretroviral-naive asymptomatic HIV-sero-positive persons. Pentoxifylline was well tolerated, and in most patients it temporarily increased CD4 and CD8 cell counts, production of mitogen-stimulated IL-2, LT, TNF-α, and IFN-γ, and production and proliferation of antigen-stimulated IL-2. Pentoxifylline temporarily reduced mitogen-stimulated ACD and plasma viremia.

Changes in CD4 and CD8 cell counts were marginal and temporary, yet the differences observed between pretherapy values and those between weeks 4 and 12 were promising (median no. of CD4 cells, 83/μL; median no. of CD8 cells, 270/μL). A slightly more robust effect (peaking at weeks 4–12) was observed when in vitro susceptibility to ACD was analyzed. The data suggest that CD4 and CD8 cell counts and in vitro susceptibility to ACD by whole PBMC might be inversely correlated parameters. Pentoxifylline stimulated production of IFN-γ, IL-2, TNF-α, and LT more than production of IL-10 and thus could be considered as a preferential stimulator of type 1 cytokine, TNF-α, and LT secretion.

The increase in TNF-α production is in contrast with previous studies showing that pentoxifylline down-regulates TNF-α production in vitro and in vivo [3, 6, 9]. Navarro et al. [6] reported that addition of pentoxifylline to HIV-infected PBMC cultures reduces TNF-α concentration and antigen-stimulated T cell proliferation. The contrast between those results and ours can be explained because the in vitro concentration of pentoxifylline utilized by Navarro et al. was 50- to 100-fold higher than the serum concentration of pentoxifylline in patients treated with the doses we used. Pentoxifylline was reported also to reduce TNF-α production in patients. This dis-
crepancy may be explained in different ways: We analyzed asymptomatic HIV-seropositive persons, whereas previous studies analyzed pentoxifylline-induced modification of TNF-α production in AIDS [6], tumor [10], or malaria patients [11]; previous studies selected HIV-seropositive patients (undergoing antiretroviral therapy) with low CD4 cell counts and high serum concentrations of TNF-α; others analyzed LPS-stimulated TNF-α production (production mainly by cells of the monocyte/macrophage lineage), whereas we measured phytohemagglutinin-stimulated TNF-α production (production by whole peripheral blood lymphocytes).

Pentoxifylline improved antigen-stimulated IL-2 production and proliferation more robustly than it improved CD4 cell counts. This observation reinforces the idea that CD4 cell count and T helper cell function are independent variables. Of interest, antigen-stimulated IL-2 production was defective in most patients. This is similar to results reported when the incidence of T cell defects was analyzed in a larger cohort of HIV-seropositive, asymptomatic patients [12]. These data also confirm that restoration of the ability to produce IL-2 upon antigen stimulation can be observed independently of dramatic changes in CD4 cell counts in HIV-seropositive persons undergoing therapy [13].

Pentoxifylline had a significant effect on reduction of HIV plasma viremia in most patients; this effect was observed despite an increase in TNF-α production. Thus, in agreement with recently published data [6], pentoxifylline can down-regulate HIV replication independently of the TNF-α-mediated effect on activation of NF-κB [6]. Reduction of plasma viremia could be secondary to the strengthening of cell-mediated immunity as implied by the increase in mitogen-stimulated type 1 cytokine production and the improvement in antigen-stimulated
IL-2 production and proliferation. Support of this hypothesis stems from the observation that IFN-γ, the concentration of which increased in our patients, has an antagonistic effect on tat-induced transactivation of HIV by long terminal repeats [14]. Reduction in plasma viremia could also be related to an increase in CD8 T lymphocytes, as CD8 cells can down-regulate HIV expression via antiviral soluble factor(s) (reviewed in [15]).

In summary, pentoxifylline can temporarily influence immunologic and virologic parameters. The reason(s) for this temporary influence could include the following: biotransformation in the liver by drug-induced enzymes, peripheral catabolism by lymphocyte, and neutralization by specific antibodies or soluble receptors. These data suggest a role for pentoxifylline, in association with antiretroviral compounds, as an immunomodulator in the therapy of HIV infection. Because the results are based on a small group of HIV-seropositive persons and because no untreated patients were analyzed in parallel with the pentoxifylline-treated ones, further studies are necessary to clarify the mechanisms of action of pentoxifylline and the best way to utilize this compound in the treatment of HIV infection.

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References