Rapid Communication

Pentoxifylline (Trental) Decreases the Replication of the Human Immunodeficiency Virus Type 1 in Human Peripheral Blood Mononuclear Cells and in Cultured T Cells

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Pentoxifylline (Trental), used routinely for the treatment of intermittent claudication, has been shown previously to decrease the levels of tumor necrosis factors-α (TNF-α) RNA in cancer patients and to lead to a general improvement of well being. Increased TNF-α levels have been observed not only in cancer patients but also in cachectic patients with the acquired immunodeficiency syndrome (AIDS), and TNF-α is known to increase the expression of the human immunodeficiency virus type 1 (HIV-1) via activating its long terminal repeat (LTR). Moreover, TNF-α decreases the therapeutic efficacy of zidovudine (AZT). Here we show a significant decrease in HIV-1 replication by pentoxifylline in infected human peripheral blood mononuclear cells. The reduction was proportional to the downregulation of expression of a reporter gene, the bacterial gene for chloramphenicol acetyl transferase, linked to the HIV-1 LTR in human monocytoid cells. We conclude that patients with AIDS may benefit from pentoxifylline treatment because of its blockage of TNF-α-mediated HIV-1 upregulation, from increased efficacy of AZT, and also from improvement in TNF-α-induced cachexia.

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MATERIALS AND METHODS

Cells and virus. PBMC, obtained by Ficoll-Hypaque gradient centrifugation of blood from HIV-1-seronegative individuals, were cultured in RPMI-1640 supplemented with 20% fetal calf serum (FCS), penicillin, streptomycin, and L-glutamine. After overnight stimulation with 15 μg/mL of concanavalin A, the cells were maintained in 10 U/mL of recombinant interleukin-2 (IL-2; Genzyme, Boston, MA). The monocytoid human cell line U38, derived from U937 cells and containing integrated copies of the HIV-1 LTR ligated to the chloramphenical acetyl transferase (CAT) gene, was obtained from Dr B. Felber (National Cancer Institute, Frederick, MD). U38 and human Jurkat cells (a CD4+ T-cell line) were grown in RPMI-1640 supplemented with 10% FCS, penicillin, streptomycin, and L-glutamine. HIV-1 was produced in Jurkat cells. During the log phase of growth, cell-free supernatant was harvested, standardized for reverse transcriptase (RT) activity,4 and frozen in aliquots at −70°C. Pentoxifylline, (a gift from Hoechst-Roussel Pharmaceuticals, Inc, Somerville, NJ) was prepared fresh for each experiment in deionized water and filter sterilized. Chronically HIV-1–infected Jurkat cells were prepared by transfection of the proviral clone pHXB27 and culture of the transfectants for over 1 year. These cells have a markedly reduced frequency of syneytium formation.

Downregulation of TNF-α by pentoxifylline in normal human PBMC. PBMC were treated at 37°C for 3 hours with 10 μg/mL LPS with various concentrations of pentoxifylline in RPMI-1640 supplemented as above. Total RNA was extracted according to Chirgwin et al., and TNF-α RNA levels were determined by hybridization with a 400-bp TNF-α probe radioactively labeled by random-priming (Boehringer Mannheim, Indianapolis, IN), using standard conditions. The TNF-α probe was generated by polymerase chain reaction (PCR) amplification of a TNF-α cDNA sequence using the oligonucleotides 5’-TTGTAGCAAACCCT-CAAGCT-3’ (upstream) and 5’-TGATCACAAGTGAGACCTGC-3’ (downstream). Filters were washed for 10 minutes at room temperature in 2X SSC (1X SSC, 0.15 mol/L sodium chloride, 0.015 mol/L sodium citrate, pH 7.0), 1% sodium dodecyl sulfate (SDS), followed by 10 minutes at room temperature in 0.5X SSC, 1% SDS, and two washes of 15 minutes each at 65°C in 0.2X SSC, 1% SDS, followed by autoradiography. Appropriate sample loading was verified by rehybridization of stripped filter with a reference probe.

β-actin probe. The autoradiograms were scanned with an LKB2222-020 Ultroscan XL Laser Densitometer (LXB Instruments, Inc, Houston, TX).

**Inhibition of HIV-1 replication by pentoxifylline.** PBMC or Jurkat cells were pretreated with various concentrations of pentoxifylline for 4 hours, after which HIV-1 (10^4 cpm units of RT activity) was added. The cells remained in the appropriate drug concentrations for 7 days. Earlier experiments had shown that high levels of RT could be obtained between days 7 to 10 postinfection in untreated cells, and analysis on day 7 was chosen to ensure a high signal-to-noise ratio. On day 7, cell viability was tested by trypan-blue staining, and RT activity was measured in cell-free supernatants.

**Downregulation of gene expression mediated by the HIV-1 LTR.** U38 cells were cultured in the presence or absence of 10 ng/mL phorbol 12-myristate 13-acetate (PMA). Two hours later, the cells were washed once in phosphate-buffered saline (PBS) and cultured in the presence or absence of pentoxifylline. On day 6, cell viability was tested by trypan-blue staining, and CAT activity was measured as described after standardizing the cell extracts for protein content.

**RESULTS**

Pentoxifylline lowered TNF-α RNA levels in LPS-simulated PBMC in a dose-dependent manner (Fig 1). At concentrations of 100 μmol/L and 500 μmol/L, pentoxifylline decreased TNF-α RNA levels (normalized to β-actin) by 32% and 74%, respectively.

Pentoxifylline decreased the replication of HIV-1 in human PBMC in a dose-dependent fashion (Fig 2). The therapeutic index, as defined by the ratio of the 50% cell growth inhibitory concentration (LD₅₀) to the 50% virus inhibitory concentration (IC₅₀), was estimated to be 5.6. At concentrations of 25 μmol/L and 250 μmol/L, HIV-1 replication as measured by RT activity in tissue culture supernatants was decreased by 25% and 70%, respectively, with little cytotoxicity. A similar level of reduction was obtained by Poli et al in experiments with TNF-α antibodies that decreased the HIV-1 RT activity by 75% in tissue-culture supernatants from PMA-stimulated U1 and ACH-2 cells.

In acutely infected Jurkat cells, a dose-dependent decrease of HIV-1 replication, as measured by RT activity in the supernatant, was noticed in the absence of significant cytotoxicity (Fig 3A). Because a level of 50% inhibition was not reached, and no cytotoxicity was noted, an estimate for the therapeutic index can not be given. In a pilot study, this decrease in HIV-1 replication was accompanied by a similar decline in TNF-α RNA levels in the same cell populations (data not shown). Untreated Jurkat cells expressed low levels of TNF-α RNA, a finding in agreement with work published earlier.

Because TNF-α is known to upregulate HIV-1 expression by inducing NF-κB binding factors, we reasoned that blocking TNF-α production may decrease gene expression mediated by the HIV-1 LTR. This hypothesis was tested in U38 cells, a monocytoid clone of U937 cells containing a stably integrated CAT gene linked to the HIV-1 LTR. Treatment of these cells with PMA led to a 10-fold stimulation of CAT activity. In PMA-stimulated cells, increasing concentrations of pentoxifylline led to a
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Fig 3. Differential effect of pentoxifylline on HIV-1 replication in acutely versus chronically infected Jurkat cells. (A) Jurkat cells were pretreated with various concentrations of pentoxifylline. Four hours later HIV-1 was added to the medium. RT activity (○) present in the supernatants of both groups was determined 7 days later. Parallel experiments were set up to determine the cytotoxicity of the drug (●). (B) Jurkat cells chronically infected with HIV-1 were cultured in the presence of various concentrations of pentoxifylline. The percent residual activity, as compared with untreated controls, is shown as a function of pentoxifylline concentration. This figure is a representative example of three independent studies in which each data point was obtained from duplicate samples.

Fig 4. Pentoxifylline down-regulates gene expression mediated by the HIV-1 LTR. U38 cells were cultured in the presence (▲, ▼) or absence (●, ○) of PMA according to Materials and Methods. Two hours later, different concentrations of pentoxifylline were added, and 6 days later, CAT activity (——) and cell growth (······) were measured. This figure is a representative example of two independent studies.

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significant decrease in CAT activity, the extent of which paralleled the inhibition of HIV-1 production seen in the experiments in human PBMC described above (Fig 4). Because the cytotoxic effects did not reach 50%, an estimate for the therapeutic index cannot be given.

DISCUSSION

Our data show that (1) pentoxifylline treatment of acutely infected human PBMC and Jurkat cells decreases HIV-1 replication; (2) in monocytoid U38 cells, HIV-1 LTR-controlled expression of a reporter gene is downregulated by pentoxifylline treatment; (3) in LPS-treated human PBMC, pentoxifylline decreased TNF-α RNA; and (4) in pilot experiments, the decrease in HIV-1 replication was parallel to decreases in TNF-α RNA levels. Of note is that the downregulation of HIV-1 replication by pentoxifylline was noticed at concentrations as low as 25 μmol/L that are achievable in patients, as shown in a recent phase I clinical trial involving cancer patients administered escalating doses of pentoxifylline.

We postulate that pentoxifylline interferes with HIV-1 replication indirectly by blocking the synthesis of cytokines, most notably TNF-α. In this context, it is interesting to note that pentoxifylline only decreased HIV-1 replication in acutely infected Jurkat T cells, but not in the chronically infected line. This differential effect of pentoxifylline may be explained by the induction of as yet unknown cytokines following acute infection of Jurkat cells by HIV-1. In contrast, chronically infected cells may have lost their sensitivity to pentoxifylline because they no longer express cytokines primarily induced by acute viral infection. Such cytokines, especially TNF-α, may play an important role in augmenting HIV-1 replication and accelerating disease progression. Within a few hours after binding of HIV-1 virions to CD4 receptors of monocyte/macrophages, TNF-α is induced, a process shown not to require viral replication. In cocultivation assays, peripheral blood B lymphocytes from HIV-1-seropositive individuals with hypergammaglobulinemia increased HIV-1 production via secretion of TNF-α and IL-6, in contrast with B lymphocytes of normal donors. However, B cells of the latter could be induced in vitro to release TNF-α. Thus, B lymphocytes of HIV-infected individuals with hypergammaglobulinemia, a condition seen frequently in this population, are capable of stimulating HIV-1 replication.

Many AIDS patients have elevated levels of TNF-α, which may contribute to the clinical symptoms such as
fever, somnolence, cachexia, and, possibly, nervous system injuries.\(^{1,27}\) In AIDS patients, the etiology for increased TNF-\(\alpha\) may be several fold: HIV-1 infection of mononuclear phagocytes per se can induce TNF-\(\alpha\) production, most probably independent of HIV-1 replication. This, in turn, augments HIV-1 replication, resulting in a positive feedback loop. Furthermore, the B-cell population of otherwise asymptomatic HIV-1-seropositive individuals with hypergammaglobulinemia produce higher levels of TNF-\(\alpha\). Lastly, opportunistic infections may lead to increased TNF-\(\alpha\) production. Blocking the reciprocal stimulation between TNF-\(\alpha\) and HIV-1 replication with pentoxifylline treatment may lower the viral burden, but also by ameliorating TNF-\(\alpha\)-induced cachexia and other symptoms. Moreover, TNF-\(\alpha\) has been shown to decrease the therapeutic efficacy of zidovudine (AZT) as well as other nucleoside analogues;\(^{28}\) thus, pentoxifylline may act synergistically with various nucleoside analogues by inhibiting TNF-\(\alpha\) production. In conclusion, the clinically approved and widely used pentoxifylline may exert significant benefits for the treatment of HIV-1 infection and its sequelae. Controlled trials will be needed to demonstrate this postulated clinical effectiveness.

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