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European Journal of Pharmacology 325 (1997) 81–84

ejp

Short communication

Pentoxifylline decreases brain levels of platelet activating factor in murine AIDS

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Received 24 February 1997; accepted 11 March 1997

Abstract

Tumor necrosis factor-α (TNF-α) and platelet-activating factor (PAF) have been implicated in the pathogenesis of human immunodeficiency virus (HIV)-associated encephalopathy. The effects of pentoxifylline on brain PAF levels were examined in mice infected with the LP-BM5 murine leukemia virus (MuLV). Seven weeks after viral inoculation, significant increases in serum TNF-α and brain PAF levels were observed. One week of treatment with pentoxifylline initiated 6 weeks postinfection significantly reduced both serum TNF-α and brain PAF levels. A significant positive correlation was observed between the levels of these substances ($r = 0.62$; $P < 0.01$). This study demonstrates that pentoxifylline treatment was effective in decreasing the levels of TNF-α in the serum and PAF levels in the brain of mice infected with the LP-BM5 MuLV.

Keywords: AIDS (acquired immunodeficiency syndrome); LP-BM5 murine leukemia virus; Encephalopathy; PAF (platelet-activating factor); Pentoxifylline; TNF-α (tumor necrosis factor-α)

1. Introduction

Mice infected with the LP-BM5 murine leukemia virus (MuLV) develop an immunodeficiency syndrome that has been termed murine acquired immunodeficiency syndrome (MAIDS) (Morse et al., 1992) and an encephalopathy whose manifestations are reminiscent of those described in humans infected with human immunodeficiency virus-1 (HIV-1) (Sei et al., 1992, 1996a). The central nervous system (CNS) abnormalities in MAIDS include: (1) impaired spatial learning and memory as characterized in the Morris water maze (Sei et al., 1992); (2) increased brain levels of the neuroactive agents quinolinic acid and platelet-activating factor (PAF) (Sei et al., 1996a; Nishida et al., 1996); and (3) astrocytic and microglial activation with microglial nodule formation (Kustova et al., 1997). In addition, 3′-azido-2′,3′-dideoxythymidine (AZT) has been found to reverse some of the cognitive deficits and neurochemical changes in mice with MAIDS (Sei et al., 1996a). These findings suggest that LP-BM5 MuLV-infected mice may be a useful model for investigating the molecular mechanisms responsible for the encephalopathy associated with retrovirus-induced immunodeficiencies.

Because HIV-1 is not neuronotropic, it has been proposed that neurotoxins produced by HIV-1-infected macrophages and activated glia are responsible for the neuronal lesions underlying HIV encephalopathy (Genis et al., 1992). A variety of potential neurotoxins have been implicated in the development of this syndrome, including tumor necrosis factor-α (TNF-α) and PAF. Studies performed in vitro indicate that co-culturing HIV-infected macrophages with astroglia results in elevated levels of TNF-α, interleukin-1β and PAF in the culture medium (Genis et al., 1992). Consistent with this in vitro finding, brain levels of both TNF-α (Wesselingh et al., 1993) and PAF (Gelbard et al., 1994) are increased and positively correlated with the severity of dementia in patients infected with HIV-1. These findings suggest that TNF-α and PAF may contribute to the neuropathogenesis of HIV-associated encephalopathy.

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PII S0014-2999(97)00047-2

Based on these findings, we hypothesize that pharmacological interventions which disrupt the neurotoxic actions of these factors may be useful in ameliorating HIV encephalopathy. The methylxanthine pentoxifylline suppresses TNF-α production at the transcriptional level, probably via interfering with nuclear factor-kappa B (NF-κB) induction (Tozawa et al., 1995), and significantly lowers serum TNF-α levels in patients with AIDS (Dezube et al., 1993). The present study employed an animal model of retrovirus-associated encephalopathy (the LP-BM5 MuLV-infected mouse), which has elevated serum TNF-α levels, to examine the effects of pentoxifylline treatment on serum TNF-α concentrations and their influence on the CNS levels of the potential neurotoxin, PAF.

2. Materials and methods

2.1. Infection of mice with LP-BM5 MuLV

C57BL/6J (B6) mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA), and housed according to NIH-AAALAC guidelines with free access to food and water. The mice were inoculated with 0.1–0.2 ml of LP-BM5 MuLV mixed virus stocks prepared from the G6 clone of chronically infected SC-1 cells. These stocks contain nonpathogenic ecotropic and mink cell focus-inducing MuLV and a replication-defective genome (BM5d) which is the critical component for disease induction.

2.2. Pentoxifylline administration

Six weeks after virus inoculation, mice were anesthetized with pentobarbital (50 mg/kg, i.p.) and implanted subcutaneously with osmotic mini-pumps (1.0 μl/h flow rate, Model 2001, ALZA, Palo Alto, CA, USA). These pumps delivered 50 mg/kg per day of pentoxifylline (Sigma, St. Louis, MO, USA) or saline for 7 days.

2.3. Quantitation of PAF and TNF-α

Seven days after mini-pump implantation, mice were anesthetized with pentobarbital. Blood was collected from the retro-orbital sinus with capillary tubes, centrifuged and the serum was isolated. Animals were perfused with saline prior to the collection of tissue samples. Brain regions (cerebral cortex and hippocampus) and spleen samples were placed into polypropylene tubes, frozen on solid CO_2, then stored at −80°C until analyzed. At the time of analysis, tissue samples were homogenized in ethanol/water (1:1, v/v) for PAF, or 100 mM NaCl/50 mM Tris-HCl, pH 7.5, containing 1.0% Nonidet P-40, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 25 μg/ml *p*-nitrophenylguanidinobenzolate, 2 mM EDTA and 1 mM sodium orthovanadate for TNF-α measurement. PAF levels were assayed using gas chromatograph followed by negative-chemical ionization mass spectrometry, modified to utilize a 15-mm DB-17 (J&W Scientific, Folsom, CA, USA) capillary column (Nishida et al., 1996). TNF-α levels were measured using a commercially available enzyme-linked immunoassay kit (Genzyme, Cambridge, MA, USA).

2.4. Statistical analysis

Individual comparisons were performed using one- or two-way analyses of variance (ANOVA), followed by Fisher's protected least significant difference test. The

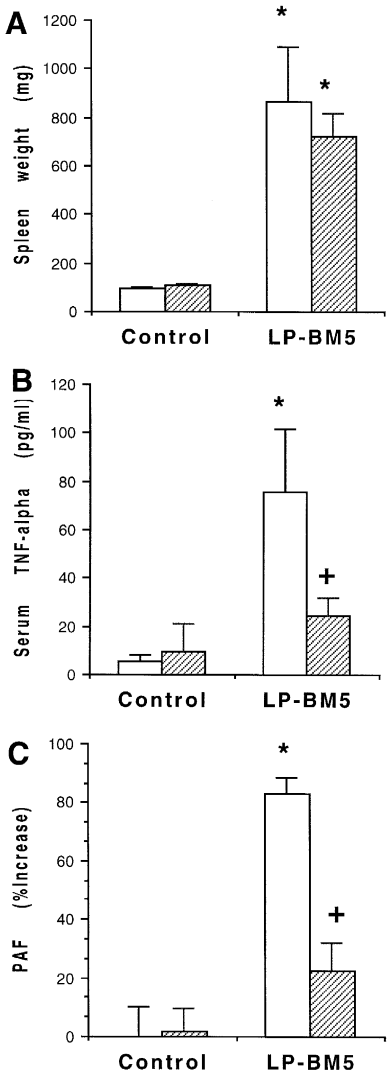

Fig. 1. Effects of pentoxifylline on spleen weight (A), serum TNF-α (B), and brain PAF (C) levels. Osmotic mini-pumps were implanted to administer pentoxifylline (50 mg/kg) for 7 days starting at 6 weeks after infection. Data indicate mean ± S.E.M (4–6 animals per group). A and B: * $P < 0.01$, and C: * $P < 0.0001$, compared to vehicle-treated and uninfected controls ($n = 4$) (Fisher's protected least significant difference test following ANOVA). B: + $P < 0.05$, and C: + $P < 0.0001$, pentoxifylline-treated (filled bar; $n = 6$) versus vehicle-treated LP-BM5 MuLV-infected animals (open bar; $n = 6$). Note that blood was flushed out of the brain by perfusion with saline prior to sample collection.

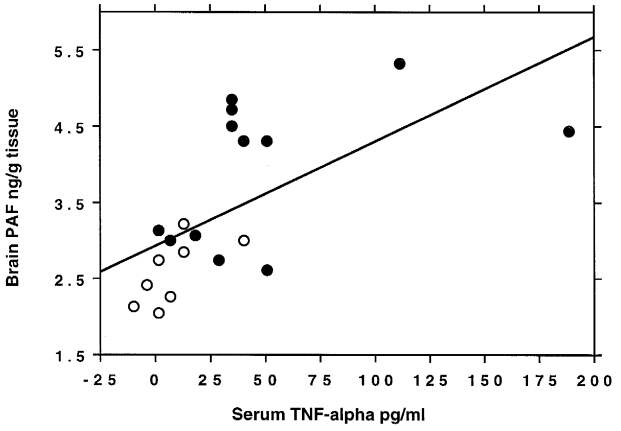

Fig. 2. Regression analysis of brain PAF and serum TNF-α levels ($n = 20$, $r = 0.623$). Closed circle indicates LP-BM5 MuLV-infected animals.

degree of correlation between serum TNF-α and CNS PAF levels was determined by Fisher's r to z correlation coefficient test. The level of statistical significance was defined as $P < 0.05$ for all tests.

3. Results

The administration of 50 mg/kg per day of pentoxifylline for 1 week beginning 6 weeks after LP-BM5 MuLV inoculation had no significant effect on the progression of the peripheral immune disease as indicated by the lack of change in spleen weights (Fig. 1A). Serum TNF-α levels increased >12-fold in mice 7 weeks after viral inoculation in saline-treated subjects, and pentoxifylline treatment significantly reduced (68%, $P < 0.05$) serum TNF-α levels (Fig. 1B). Brain TNF-α levels in control and LP-BM5 MuLV-infected animals were comparable (332.0 ± 26.3 and 367.9 ± 18.2 pg/g of tissue, respectively) and were not significantly altered by pentoxifylline therapy (371.2 ± 29.4 and 332.2 ± 10.7 pg/g of tissue, respectively). In contrast, pentoxifylline administration significantly decreased (73.5%, $P < 0.0001$) hippocampal PAF levels (Fig. 1C). A significant positive correlation was found between serum concentrations of TNF-α and brain levels of PAF ($r = 0.62$, $P = 0.0026$) (Fig. 2), whereas there was no correlation between brain TNF-α and PAF levels ($r = 0.14$, $P = 0.58$).

4. Discussion

Substantial increases in the expression of TNF-α have been demonstrated in humans infected with HIV-1 (Dezube et al., 1993). Similarly, mice infected with the LP-BM5 MuLV showed substantial increases in serum TNF-α levels 7 weeks after virus inoculation. This latter observation is consistent with a previous report demonstrating the increased expression of TNF-α mRNA in the lymph nodes and spleen of LP-BM5 MuLV-infected mice (Morse et al., 1992). Major sources of peripheral TNF-α in mice infected with LP-BM5 MuLV are activated monocyte/macrophages, and to a lesser degree, B and $CD4^+$ T cells. The present study showed that pentoxifylline significantly decreased serum TNF-α levels in the LP-BM5 MuLV-infected mice. Pentoxifylline treatment had no effect on spleen weight, suggesting that this decrease in serum TNF-α levels was independent of any effect on the progression of the underlying viral infection. This finding is also consistent with clinical reports that pentoxifylline decreased serum TNF levels in patients with AIDS (Dezube et al., 1993).

PAF levels are increased 1.5–2.0-fold in the frontal cortex and hippocampus of mice infected with LP-BM5 MuLV (Nishida et al., 1996). One week of pentoxifylline treatment resulted in a significant reduction in brain PAF levels. Moreover, there was a statistically significant correlation between the levels of TNF-α in the serum and PAF levels in the CNS but not between CNS TNF-α and PAF levels. These findings suggest that the changes in brain PAF levels in these mice were not due to increased synthesis or release of CNS TNF-α. The interaction between these factors seemed to be also absent in peripheral organs since spleen PAF levels were not increased (Nishida et al., 1996) while serum TNF-α levels were over 10-fold increased in these mice. However, it may be possible that serum TNF-α is linked with brain PAF through glutamatergic activation of neurons. Thus, TNF-α could directly or indirectly increase the blood–brain barrier and activate microglia and astrocytes (Kahn et al., 1995), which may result in increases in levels of excitotoxins such as glutamate (Piani et al., 1991) and quinolinic acid (Sei et al., 1996a), thereby increasing PAF synthesis via NMDA receptors. Such a scenario is supported by our observations of prominent gliosis (Kustova et al., 1997), glutamatergic hyperactivation (Sei et al., 1996b) and the ability of the NMDA antagonist MK-801 (0.5 mg/kg/day, 1 week) to significantly reduce brain PAF levels in LP-BM5 MuLV-infected mice (Nishida et al., 1996). Finally, it should be also considered that pentoxifylline could directly affect PAF-producing cells as demonstrated in the report that pentoxifylline directly decreases PAF by increasing cellular levels of cAMP in human neutrophils (Fonteh et al., 1993).

The present study demonstrates that pentoxifylline treatment was effective in reducing PAF and serum TNF-α levels in mice infected with LP-BM5 MuLV. It is important to note that increases in brain PAF levels resulting from the LP-BM5 MuLV infection are reversible. Since both PAF and TNF-α have been implicated in the pathogenesis of the CNS manifestations of HIV (Genis et al., 1992), administration of pentoxifylline could be beneficial in delaying or reversing the development of HIV encephalopathy by suppressing synthesis of PAF and TNF-α.

However, additional studies are necessary to rigorously test this hypothesis.

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