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Modulation of human immunodeficiency virus infection by anticonvulsant drugs

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Patients with human immunodeficiency virus (HIV) infection often require treatment with anticonvulsants either for treatment of seizures or occasionally for pain control. In this study, the authors determined if the anticonvulsants phenytoin, carbamazepine, and valproate could modulate HIV replication. These drugs activated HIV replication in latently infected monocytic cells but not in latently infected lymphocytic cells at clinically relevant dosages. The activation in the monocytic cells was as a result of transactivation of HIV long terminal repeat (LTR) and could be seen at therapeutic dosages whereas no effect was seen on LTR activation in lymphocytic cells. When the drugs were used in conjunction with known transactivators of HIV LTR such as Tat and phorbol-12-myristate-13-acetate (PMA), no additive or synergistic effect was noted. Although the clinical relevance of these observations needs to be determined, these observations may suggest that monitoring of cerebrospinal fluid (CSF) viral load maybe needed in HIV-infected patients treated with anticonvulsants, because HIV-infected macrophages are important in mediating HIV dementia. *Journal of NeuroVirology* (2006) **12,** 1–4.

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Introduction

Patients with human immunodeficiency virus (HIV) infection develop a number of complications that require treatment with the anticonvulsant class of drugs. Seizures occur in the general adult population at a rate of 1% to 2%; however, nearly 10% of HIV-infected patients develop seizures. Seizures may occur as a consequence of HIV infection alone, due to opportunistic infections (e.g., cerebral toxoplasmosis, cryptococcal meningitis, or central nervous system [CNS] tuberculosis), primary CNS lymphomas, renal failure from HIV nephropathy, substance abuse (Wong *et al*, 1990), or treatment with nucleoside analogs (Wong *et al*, 1990). It is also conceivable that an individual with a preexisting seizure disorder may contract HIV infection, therefore requiring continuation of anticonvulsant therapy. Unless a reversible precipitating factor can be identified, it has been recommended that HIV-infected individuals receive anticonvulsant therapy after their first seizure. Peripheral neuropathy, causing a burning pain in the feet, may occur due to HIV infection alone or as an adverse effect of antiretroviral drugs, affects upwards of 40% of patients, and is often treated with various anticonvulsant agents (Wolfe and Barohn, 2002). Other painful conditions, such as postherpetic neuralgia and headaches, occur in nearly 40% of HIV-infected patients and may also respond to anticonvulsants (Mirsattari *et al*, 1999). Additionally, major psychiatric disorders, such as depression, bipolar disorders, and mania, are common in the HIV-infected population and are typically refractory to traditional therapies, thus requiring treatment with anticonvulsants (Halman *et al*, 1993). Anticonvulsants may also have neuroprotective properties and hence have been suggested for treatment of HIV dementia (Dou *et al*, 2003) and neuropathies (Cui *et al*, 2003)

Protease inhibitors are potent inhibitors of hepatic cytochrome P450, and hence can lead to toxic levels of those anticonvulsants that are metabolized by this pathway (Berbel Garcia *et al*, 2000). Conversely, induction of hepatic cytochrome P450 by

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carbamazepine has been implicated in accelerating the metabolism of antiretroviral drugs and the emergence HIV-resistant strains (Hugen *et al*, 2000). Although these drug-drug interactions in HIV-infected populations have received much deserved attention, the possibility that anticonvulsants may directly affect HIV replication in these patients has not been studied. To determine the interactions between HIV replication and anticonvulsants, we chose to study the three of the most widely available anticonvulsants worldwide, phenytoin, carbamazepine, and valproate.

Results

Effect of anticonvulsants on HIV-infected lymphocytes and monocytes

No significant effect of any of the anticonvulsants was noted on HIV replication in the latently infected lymphocytes when treated with the drugs alone. Both, Tat and phorbol-12-myristate-13-acetate (PMA) were able to induce HIV replication in these cells as determined by p24 release in the culture supernatants (Figure 1). To determine if the drugs may have an effect on actively replicating virus, we incubated the cells with the anticonvulsants phenytoin, carbamazepine, and valproate. None of the drugs had a significant effect on HIV replication in the lymphocytic cells in the presence or absence of Tat and PMA at all dosages tested (Figure 1A). Conversely, all the drugs induced HIV replication in the latently infected monocytic cells at therapeutic dosages (Figure 1B). However, no synergism of additive effect was noted with Tat and PMA.

Effect of anticonvulsants on HIV-LTR activation on lymphocytes and monocytes

To determine if the effects of the drugs maybe mediated via the noncoding regulatory region of HIV (long terminal repeat, LTR), we incubated the drugs with cell lines stably transfected with LTR linked to green fluorescence protein (GFP). Consistent with the p24 release results, we noted that none of the drugs were able to cause LTR activation in the lymphocytic cells when incubated alone or in the presence of Tat and PMA (Figure 2A). In monocytic cells, consistent with the p24 results, each of the drugs activated LTR by themselves even at subtherapeutic dosages and caused further activation in the presence of Tat + PMA (Figure 2B). No effect of the drugs was noted when incubated in the presence or absence of Tat + PMA on LTR in an epithelial cell line (Hela-CD4-LTR-β-galactosidase) (data not shown), suggesting the drugs effects are cell type specific. Each of the drugs effects occurred in a dose-responsive manner and similar effects were noted when analyzed at 24 or 48 h (data not shown).

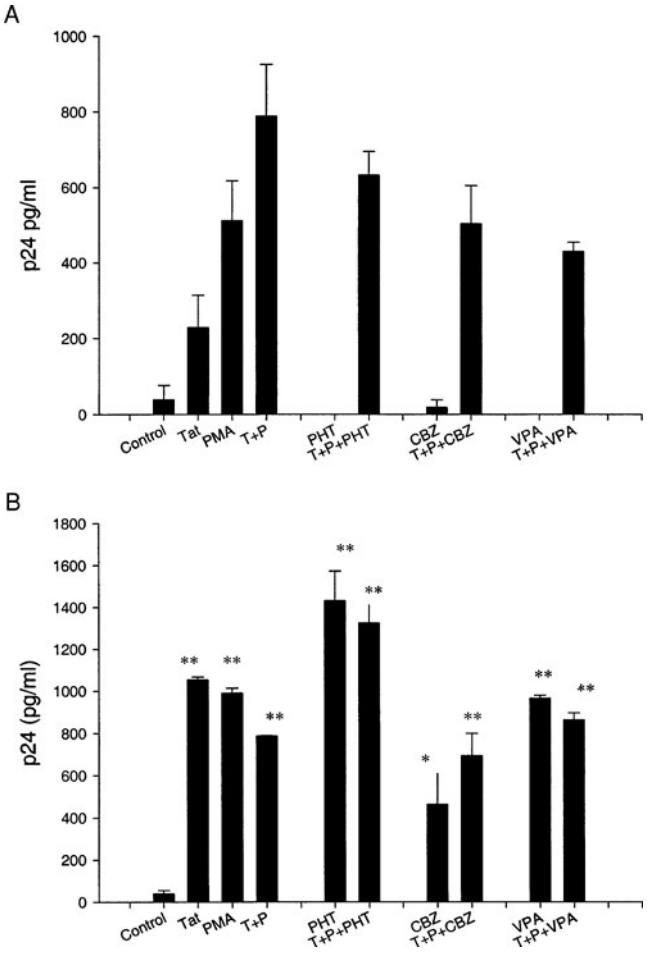

Figure 1 Effect of anticonvulsant drugs on HIV replication. (**A**) Latently infected lymphocytic cells (J-1) were incubated with Tat (200 nM) or PMA (3 μM), alone or together Tat + PMA (T + P). Carbamazepine (CBZ 8 μg/ml), phenytoin (PHT 15 μg/ml), valproate (VPA 75 μg/ml) were incubated alone or in combination with T + P. Forty-eight hours later, cell culture supernatants were analyzed for p24 antigen by ELISA. There was no significant difference between the untreated controls and the anticonvulsant-treated cultures; and no significant difference between the T + P–treated cultures in the presence or absence of the anticonvulsant drugs. (**B**) Latently infected monocytic cells (U-1) were similarly treated. Significant p24 production was noted with each of the anticonvulsants. $^{*}P < .05$, $^{**}P < .01$ compared to controls. Data represents mean and SE from at least three different experiments done in triplicates.

Discussion

Our study reveals that anticonvulsants may regulate HIV replication in monocytes. Importantly, HIV replication was induced in monocytic cells by the drugs at concentrations within the therapeutic range. Macrophages are the major cell type within the brain and dorsal root ganglia infected with HIV and these cells play an important role in mediating HIV dementia and probably HIV peripheral neuropathy as well (Nath, 2002). Activation of HIV infection in this cell type could lead to increased HIV viral load in nervous system tissue despite control of HIV replication in the peripheral blood in patients treated with

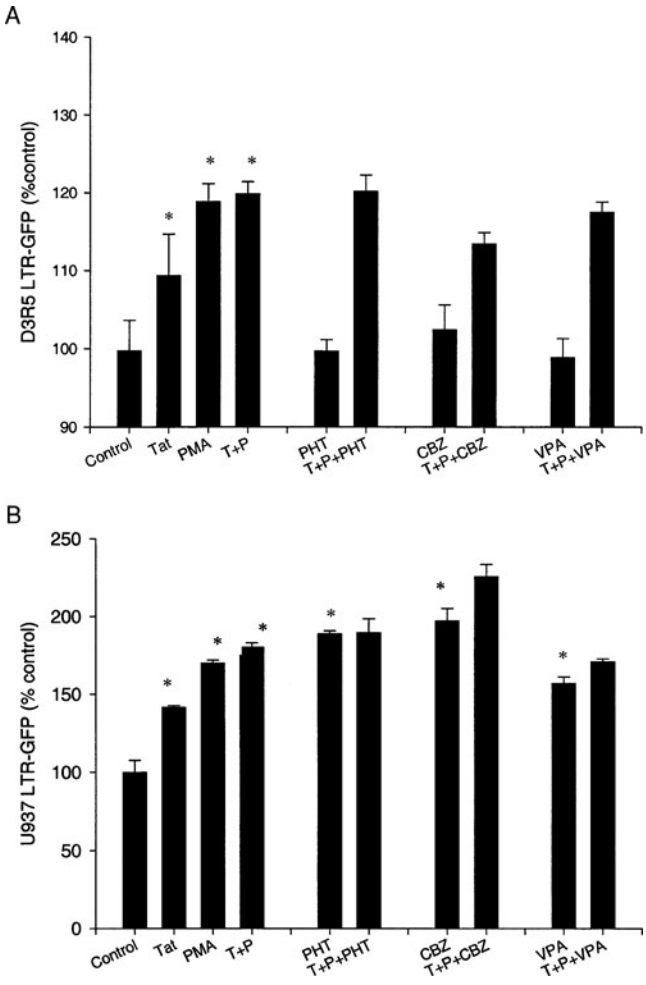

Figure 2 Effect of anticonvulsants on LTR activation. (**A**) Lymphocytic cells stably transfected with LTR-GFP (D3R5-LTR-GFP) were incubated with Tat (200 nM) or PMA (3 μM) alone or together (T + P) in the presence or absence of carbamazepine (CBZ 8 μg/ml), phenytoin (PHT 15 μg/ml), valproate (VPA 75 μg/ml). (**B**) Monocytic cells stably transfected with LTR-GFP (U937-LTR-GFP) were incubated with Tat (200 nM) or PMA (3 μM) alone or together (T + P). CBZ 8 μg/ml, PHT 15 μg/ml, or VPA 75 μg/ml were incubated alone or in combination with T + P. Twenty-four hours later the cells were analyzed by a florescent plate reader. Data represent mean and SE from three experiments done in replicates of eight and expressed as percentage of control values. $^{*}P < .05$ compared to control; $^{\#}P < .05$ compared to T+P.

antiretroviral drugs, a phenomenon termed "viral escape" (Stingele *et al*, 2001). Factors involved in viral escape are not fully understood; however, it may be possible that patients on anticonvulsant drugs may be at risk of viral escape. In contrast to anticonvulsant drugs, most antiretroviral drugs have poor penetration into the brain and cerebrospinal fluid (CSF) (Letendre *et al*, 2004), hence careful monitoring of CSF viral load may be essential in HIV-infected patients being treated with anticonvulsants. Similarly, HIV-infected macrophages play an important role in mediating a distal symmetric peripheral neuropathy, hence anticonvulsants may negatively impact its pathogenesis.

Our study examined only a select number of anticonvulsants drugs. However, carbamazepine, phenytoin, and valproate are some of the most commonly used drugs worldwide especially in populations that face the largest burden of HIV infection. No major differences were found between the drugs tested. The mechanism of HIV induction by anticonvulsants in monocytic cells seems to involve LTR activation and is supported by a previous studies where valproate was also shown to cause enhanced HIV replication (Moog *et al*, 1996; Witvrouw *et al*, 1997). However the mechanisms by which these drugs lead to LTR transactivation remains unknown. Anticonvulsant drugs in general alter neuronal membrane excitability by acting on ion channels. It is possible that similar receptor-mediated events result in activation of secondary signaling pathways, eventually resulting in LTR activation, but this possibility requires further investigation. Several new anticonvulsants drugs have become available in recent years and it may be necessary to identify those drugs that do not alter HIV replication in mononuclear cells.

Materials and methods

HIV antigen assay

Lymphoid (J1) and monocytic (U1) cells latently infected with HIV (Perez *et al*, 1991) were treated in microtiter plates (10^5 cells/well) with carbamazepine (25 ng/ml to 8 μg/ml) sodium valproate (17 ng/ml to 75 μg/ml), or phenytoin (25 ng/ml to 15 μg/ml) for 48 h in the presence or absence of the HIV transactivating protein Tat (250 nM) and/or PMA (10 mM). Carbamazepine was dissolved in ethanol and then mixed with Locke's buffer (pH 7.2) or medium. The reviewer had right concerns with phenytoin's solubility. Phenytoin was dissolved in deionized double distilled water adjusted with 10 nM NaOH and then mixed with Locke's buffer (pH 7.2) or medium. Sodium valproate and vigabatrin are both soluble in pH-adjusted water. No precipitation was noted at the time the drugs were added or during any time of the experiments. p24 antigen (HIV core protein) was measured in culture supernatants by a sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Beckman Coulter, Fullerton, CA). Mean ± SE were calculated and analyzed by analysis of variance (ANOVA).

LTR transactivation assay

U937 macrophage cells were transfected with a HIV-LTR-GFP plasmid using DMRIE-C reagent (GibcoBRL) according to manufacturer's protocol. Stably transfected cells were selected in neomycin and used for further analysis. U937-LTR-GFP and a lymphocytic cell line D3R5-LTR-GFP (Dorsky and Harrington, 1999) were incubated in microtiter plates (10^5 cells/well) with each of the anticonvulsants in

the presence or absence of Tat and/or PMA as described above. Cells were analyzed at 24 and 48 h by a fluorescent plate reader and calculated as mean ± SE from percentage of control florescence units. No auto florescence was noted with any of the drugs. Data were analyzed by ANOVA.

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