Molecular and Cellular Neuroscience 65 (2015) 11–20

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

GSK3β-activation is a point of convergence for HIV-1 and opiate-mediated interactive neurotoxicity

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ARTICLE INFO

Article history:
Received 2 September 2014
Revised 22 December 2014
Accepted 19 January 2015
Available online 20 January 2015

Keywords:
NeuroAIDS
HIV-1
Morphine
Neurodegeneration
Synaptodendritic injury
GSK3β

ABSTRACT

Infection of the CNS with HIV-1 occurs rapidly after primary peripheral infection. HIV-1 can induce a wide range of neurological deficits, collectively known as HIV-1-associated neurocognitive disorders. Our previous work has shown that the selected neurotoxic effects induced by individual viral proteins, Tat and gp120, and by HIV$^+$ supernatant are enhanced by co-exposure to morphine. This mimics co-morbid neurological effects observed in opiate-abusing HIV$^+$ patients. Although there is a correlation between opiate drug abuse and progression of HIV-1-associated neurocognitive disorders, the mechanisms underlying interactions between HIV-1 and opiates remain obscure. Previous studies have shown that HIV-1 induces neurotoxic effects through abnormal activation of GSK3β. Interestingly, expression of GSK3β has shown to be elevated in brains of young opiate abusers indicating that GSK3β is also linked to neuropathology seen with opiate-abusing patients. Thus, we hypothesize that GSK3β activation is a point of convergence for HIV- and opiate-mediated interactive neurotoxic effects. Neuronal cultures were treated with supernatant from HIV-1_{SF162}-infected THP-1 cells, in the presence or absence of morphine and GSK3β inhibitors. Our results show that GSK3β inhibitors, including valproate and small molecule inhibitors, significantly reduce HIV-1-mediated neurotoxic outcomes, and also negate interactions with morphine that result in cell death, suggesting that GSK3β-activation is an important point of convergence and a potential therapeutic target for HIV- and opiate-mediated neurocognitive deficits.

1. Introduction

Infection of the central nervous system (CNS) with human immunodeficiency virus-1 (HIV-1) occurs rapidly after primary peripheral infection (An et al., 1996, 1999b; Davis et al., 1992; Kramer-Hammerle et al., 2005). HIV-1 can induce a wide range of CNS deficits, collectively known as HIV-1-associated neurocognitive disorders (HAND); nearly 50% of HIV-1-infected individuals suffer from HAND (Fischer-Smith and Rappaport, 2005; McArthur, 2004; McArthur et al., 2003, 2005). Infected and/or activated glial and immune cells in the CNS release various viral and cellular factors that drive direct and indirect neuronal toxicity, leading to HAND (Buescher et al., 2007; Epstein and Gendelman, 1993; Gonzalez-Scarano and Martin-Garcia, 2005; Kaul et al., 2005; Kramer-Hammerle et al., 2005). The advent of combination antiretroviral therapy (cART) has reduced the severity of HAND, but the disease prevalence remains the same (Cysique and Brew, 2009; Cysique et al., 2004; Ellis et al., 2007; Robertson et al., 2007; Robinson-Papp et al., 2009; Sacktor, 2002; Sacktor et al., 2002), likely due to longer patient survival (Dore et al., 2003) and the relatively poor CNS penetrance of many antiretroviral drugs (Ellis et al., 2007; Kerza-Kwiatecki and Amini, 1999).

Drug abuse is a major risk factor for HIV infection; nearly 30% of HIV$^+$ patients have a history of drug abuse involving opiates (Bokhari et al., 2009; Robertson et al., 1994). A large fraction of HIV$^+$ patients are also exposed to opiates for treatment of AIDS-related chronic pain syndromes. Opiate drugs of abuse modulate immune function (Novick et al., 1989; Rogers and Peterson, 2003; Sheng et al., 1997), and also promote HIV-1 replication in vitro (Peterson et al., 1990, 2004). Since opiates by themselves promote outcomes involved in CNS dysfunction, such as blood–brain barrier breakdown, immune and glial cell activation, and neuronal damage (Bell et al., 2006; Hauser et al., 2005; Hu et al., 2002; Sheng et al., 1997), it can be predicted that opiate drug

Abbreviations: CNS, central nervous system; HIV-1, human immunodeficiency virus-1; HAND, HIV-1-associated neurocognitive disorders; cART, combination antiretroviral therapy; Tat, trans-activator of transcription; gp120, glycoprotein 120; GSK3β, glycogen synthase kinase-3β; MAP-2, microtubule-associated protein 2; MORs, μ-opioid receptors; VPA, valproate; p-GSK3β-S9, phospho-GSK3β-Ser9; PSD-95, postsynaptic density protein 95; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; FGFs, fibroblast growth factors; PI3K, phosphatidylinositol-3-kinase; AP-1, activator protein 1; CREB, cyclic AMP response element binding protein; NF-κB, nuclear factor-kappa B; HSF-1, heat shock factor-1; MAPK10, mitogen-activated protein kinase 10; PKR, double-stranded RNA-activated protein kinase; CDK5, cyclin-dependent kinase 5; HDAC, histone deacetylase.

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http://dx.doi.org/10.1016/j.mcn.2015.01.001
1044-7431/

abuse might exacerbate HIV-1 pathogenesis in the CNS. Our previous work has shown that certain neurotoxic effects induced by the individual HIV-1 proteins, trans-activator of transcription (Tat) and glycoprotein 120 (gp120) (Fitting et al., 2010a; Gurwell et al., 2001; Podhaizer et al., 2012; Suzuki et al., 2011; Zou et al., 2011b), and by HIV^+ supernatant (HIV^+_{sup}) (Masvekar et al., 2014), are enhanced by co-exposure to morphine, the major metabolite of heroin in the CNS (Sawynok, 1986). This mimics co-morbid neurological effects observed in opiate-abusing HIV^+ patients (Anthony et al., 2008; Bell et al., 2002; Byrd et al., 2011; Meijerink et al., 2014; Robinson-Papp et al., 2012; Smith et al., 2014). Although there is a correlation between opiate drug abuse and HAND progression, the mechanisms that underlie interactions between HIV-1 and opiates remain obscure; the main aim of this work was to identify point(s) of convergence for HIV-1 and morphine signaling in neurons.

Identified originally as a regulator of glycogen metabolism, glycogen synthase kinase-3β (GSK3β) is a central component of various signaling pathways in neurons, including those affecting neuronal plasticity, gene expression, and cell survival (Frame and Cohen, 2001; Grimes and Jope, 2001; Jacobs et al., 2012). GSK3β activity appears to be dysregulated in multiple neuropathological conditions, including Alzheimer's disease, Parkinson's disease, schizophrenia, autism, and bipolar mood disorder, and pharmacological inhibition of GSK3β is effective against some symptomatology in these diseases (Emamian et al., 2004; Frame and Cohen, 2001; Haenisch et al., 2014; Jacobs et al., 2012; Kaytor and Orr, 2002; Koistinaho et al., 2011; Kozikowski et al., 2006; Leroy et al., 2007; Schaffer et al., 2008). In previous studies, HIV-1 neurotoxicity was linked to abnormal activation of GSK3β (Crews et al., 2009; Dou et al., 2003, 2005; Everall et al., 2002; Maggirwar et al., 1999; Sui et al., 2006), GSK3β has also been linked to neuropathology seen in opiate-abusing patients (Anthony et al., 2010; Ramage et al., 2005). We therefore tested GSK3β activation as a point of convergent signaling for interactions between HIV-1 and morphine.

Both lethal and sublethal effects of $HIV^+_{sup} \pm$ morphine treatments were assessed on neuron populations, and also by time-lapse imaging of individual cells over 72 h. Valproate and small molecule GSK3β inhibitors significantly reduced HIV^+_{sup}-mediated neurotoxic outcomes. Interactions between HIV and morphine that resulted in neuronal death were also abrogated, implicating GSK3β as a mediator of specific neurotoxic events initiated by combined exposure to HIV-1 and opiates.

2. Results

2.1. HIV- and morphine-mediated GSK3β activation

GSK3β activation/inactivation was assessed at 4 h after treatments. Cell lysates were immunoblotted for phospho-GSK3β-Ser9 (p-GSK3β-S9; an inactive form of GSK3β) (Frame et al., 2001; Grimes and Jope, 2001; Jacobs et al., 2012; Kaytor and Orr, 2002), GSK3β (total GSK3β; t-GSK3β), β-catenin, and GAPDH (Fig. 1). HIV^+_{sup}, with or without morphine, significantly decreased p-GSK3β-S9 (normalized with t-GSK3β; p-GSK3β-S9/t-GSK3β; Fig. 1A), suggesting an increase in GSK3β activation. VPA significantly abrogated $HIV^+_{sup} \pm$ morphine-mediated effects. β-Catenin is a downstream target of GSK3β; active GSK3β phosphorylates β-catenin and initiates rapid ubiquitin-mediated degradation by the proteasome (Aberle et al., 1997; Jacobs et al., 2012; Kaytor and Orr, 2002). HIV^+_{sup}, with or without morphine, significantly reduced β-Catenin levels (Fig. 1B), suggesting an enhancement in GSK3β activity. VPA completely inhibited the effect of HIV^+_{sup} + morphine and partially inhibited the effect of HIV^+_{sup} alone, returning both to levels that were not different from control levels.

Significant individual or interactive effects of morphine were not detected at 4 h. Thus, based on the time-dependent effects of opiates observed by others (Dobashi et al., 2010; Xie et al., 2010), we examined HIV^+_{sup} and morphine-mediated effects at longer time intervals (Fig. 2). At all assessed time points (12, 24, and 72 h), HIV^+_{sup} significantly reduced p-GSK3β-S9. At 24 and 72 h, morphine alone also induced significant loss of p-GSK3β-S9. A significant interaction between HIV^+_{sup} and morphine was seen only at 24 h.

2.2. Role of GSK3β in HIV ± morphine-mediated cell death

Death of pre-selected neurons was observed using time-lapse imaging; cells were repeatedly imaged at 1 h intervals throughout the 72 h treatment period (Fig. 3A). Neurons exposed to morphine and/or VPA had survival values equivalent to control, while all groups treated with HIV^+_{sup} showed significantly reduced neuronal survival (Fig. 3B). Morphine significantly enhanced HIV^+_{sup}-mediated neuronal death. $HIV^+_{sup} \pm$ morphine-mediated effects were partially, but significantly, reversed by VPA co-treatment. The survival of neurons treated with 'HIV^+_{sup} + VPA' and 'HIV^+_{sup} + morphine + VPA' was not significantly different, showing that VPA effectively negated any HIV^+_{sup}-morphine interactions. Cell death was additionally confirmed using TUNEL staining (Fig. 3C). HIV^+_{sup} induced a significant increase in TUNEL(+) neurons, which was augmented by morphine co-treatment. $HIV^+_{sup} \pm$ morphine-mediated effects were partially, but significantly, reversed by VPA co-treatment. As seen for the time-lapse analyses, VPA also nullified the interactions between HIV^+_{sup} and morphine.

2.3. Role of GSK3β in HIV ± morphine-mediated changes in neuritic arborization, MAP-2 and PSD-95

Since neurite degeneration and synapse losses are the major substrate for HAND (Bellizzi et al., 2006; Ellis et al., 2007; Everall et al., 1999; Kim et al., 2008; Masliah et al., 1997) we assessed changes in neuritic arborization using MAP-2 immunostaining (Fig. 4A) followed by Sholl analysis (Fig. 4B). At 72 h, HIV^+_{sup} induced a significant reduction in neuritic arborization. There was no significant morphine interaction, and the HIV^+_{sup} effect was partially, but significantly, reversed by VPA co-treatment. Neuritic/synaptic degeneration was additionally confirmed by immunoblotting for MAP-2 and PSD-95 (Fig. 4C and D). At 72 h, HIV^+_{sup} significantly decreased MAP-2. Similar to the Sholl analysis, there was no significant morphine interaction (Fig. 4C), and VPA significantly abrogated HIV^+_{sup}-mediated loss of MAP-2. HIV^+_{sup} also induced a significant loss in PSD-95, but in this case, there was an increased loss with morphine co-treatment (Fig. 4D). VPA partially, but significantly, reversed $HIV^+_{sup} \pm$ morphine effects on PSD-95. The HIV-morphine interaction on PSD-95 levels was maintained in the presence of VPA.

Neuritic/synaptic degeneration was also assessed in cortical and hippocampal neurons (Fig. 5). In cortical neurons (Fig. 5A), HIV^+_{sup} significantly reduced MAP-2 and PSD-95, without significant morphine interactions. VPA significantly abrogated HIV^+_{sup}-mediated loss of MAP-2 and partially, but significantly, abrogated loss of PSD-95. In hippocampal neurons (Fig. 5B), HIV^+_{sup} induced a significant loss of MAP-2 and PSD-95, which was significantly augmented by morphine co-treatment. VPA significantly reversed $HIV^+_{sup} \pm$ morphine-mediated effects on MAP-2 and partially, but significantly, reversed effects on PSD-95.

2.4. Effects of small molecule GSK3β-inhibitors

The role of GSK3β activation in $HIV^+_{sup} \pm$ morphine-mediated neurotoxicity was confirmed additionally using the small molecule GSK3β-inhibitors, SB415286 or XXVI (Fig. 6), both of which have very high specificity for GSK3β compared to VPA. Both small molecule inhibitors partially, but significantly, reduced $HIV^+_{sup} \pm$ morphine-mediated neuronal death, and also abrogated HIV^+_{sup}-morphine interactions (Fig. 6A and B). Both inhibitors reversed the effect of $HIV^+_{sup} \pm$ morphine on neurite losses, although SB415286 only resulted in partial recovery (Fig. 6C).