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Adjunctive and Long-Acting Nanoformulated Antiretroviral Therapies for HIV-associated neurocognitive disorders

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Abstract

Purpose of review—This review focuses on current and future strategies to modulate neuroinflammation while reducing residual viral burden in the central nervous system (CNS). This has been realized by targeted long acting antiretroviral nano- and adjunctive therapies being developed for HIV infected people. Our ultimate goal is to eliminate virus from its CNS reservoirs and, in so doing, reverse the cognitive and motor dysfunctions seen in HIV-associated neurocognitive disorders (HAND).

Recent findings—Herein, we highlight our laboratories development of adjunctive and nanomedicine therapies for HAND. An emphasis is placed on drug-drug interactions that target both the viral life cycle and secretory pro-inflammatory neurotoxic factors and signaling pathways.

Summary—Antiretroviral therapy (ART) has improved the quality and duration of life for people living with HIV-1. A significant long-term comorbid illness is HAND. Symptoms, while reduced in severity, are common. Disease occurs, in part, through continued low-level viral replication inducing secondary glial neuroinflammatory activities. Our recent works and those of others have seen disease attenuated in animal models through the use of adjunctive and long-acting reservoir targeted nanoformulated ART. The translation of these inventions from animals to humans is the focus of this review.

Keywords

HIV-associated neurocognitive disorders; nanoformulated antiretroviral therapy; neuroinflammation; mixed lineage kinase

Introduction

While the medical treatment for HIV infection with potent combination antiretroviral therapy (ART) has reduced disease morbidities and mortalities, the prevalence of neurologic

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disease associated with viral infection has continued almost unabated [1]. Elimination of HIV-1 associated neurocognitive disorders (HAND), while important in the overall treatment strategies for HIV/AIDS, has found a secondary position in therapeutic design for several reasons. *First* and foremost is the acceptance that HAND has shifted to a more indolent disease phenotype compared to the profound dementia and motor and behavioral abnormalities that have all but vanished but were previously commonplace [2]. *Second*, the progressive encephalopathy seen in children has been virtually eliminated by ART. This has lulled people living with HIV and their healthcare providers into a mindset of the problem is gone and for "watchful waiting" in disease elimination all together. Even more telling, practitioners do not screen for HAND. This is based on the lack of awareness of subtle neurologic disease in the ART era coupled with inadequate information about its prevalence and limitations in screening tool use and consensus [3]. Though the incidence of the most severe forms of HAND has plummeted, prevalence of all forms of HAND has steadily increased with estimates that range between 40–70% in infected individuals [4], regardless of the ART regimen used and whether or not the drugs target the central nervous system (CNS) [5]. Perhaps more surprisingly, reliance on a CNS penetration score (CPE) for selection of drugs that have favorable CNS profiles (and high CPE scores) has led to the disturbing finding that high CPE scores correlate with a diagnosis of cognitive impairment in infected people [6]. Possible reasons for this include drug-CNS toxicity, patient adherence with high CPE score regimens or even selection bias based on patients with pre-existing HAND who choose such regimens [6]. Regardless, these observations, over 30 years after the AIDS epidemic started, are dispiriting and warrant a renewed push towards developing strategies that can both re-establish homeostasis between CNS immune effector cells and functional synapses as well as eliminate HIV CNS reservoirs that initiate the neuroinflammation that leads to HAND. To these ends, our recent works have sought to merge studies of adjunctive anti-inflammatory and neuroprotective therapies with new delivery schemes for ART. A mixed lineage kinase 3 (MLK3) inhibitor, URMC-099, uncovered for HAND treatments was tested in conjunction with long acting nanoformulated ART (nanoART) and was found to affect drug levels and speed viral elimination in experimental animal models of HIV/AIDS. These effects, interestingly enough, were associated with enhanced particle trafficking in macrophage recycling endosomes. These activities of URMC-099 and those of others provide the means to significantly boost efforts to clear persistent viral infection and cognitive function in HIV infected people [7].

Notwithstanding, the current review focuses on these and other current and future therapeutic strategies to achieve reduction or resolution of neuroinflammation with disease modifying outcomes for HAND with the ultimate goal of eliminating HIV from CNS reservoirs. The overarching idea is to harness novel nanomedicine combating strategies that facilitate ART virucidal activities during viral infection. Prior developed adjunctive therapies for HAND formed the foundation for these works and are reviewed elsewhere [1, 8].

Challenges to viral eradication in the CNS

One of the greatest challenges in the treatment of HIV-1 infection rests in the abilities of ART to target viral cellular reservoirs in barrier compartments. This includes the gut,

lymphoid tissues and the CNS. The latter poses a number of inherent problems: (1) hijacking pro-inflammatory signaling in macrophages/microglia despite combination ART (cART) - mediated suppression of the viral life cycle; (2) bystander toxicity to target cells such neurons and oligodendrocytes with limited or no capacity for self-renewal; (3) eradication of virus may paradoxically lead to more inflammation when the end point is cell death of reservoir populations. Since many of the deleterious substrates for HAND such as destruction of normal synaptic architecture in the CNS are associated with neuroinflammation, a focus on molecular targets such as the MLKs, and in particular MLK-3, that serve as kinase control hubs for mediating inflammation may be key to disease-modifying therapies.

Mixed lineage kinases

MLKs are mitogen activated protein kinase kinase kinases (MKKKs) with features of both serine-threonine and tyrosine kinases (hence the nomenclature “mixed lineage”) that regulate the c-Jun N-terminal kinase (JNK) mitogen activated protein kinase (MAPK) signaling cascade (Fig. 1), and also regulate the other two major MAPK pathways, p38 and extracellular signal-regulated kinase (ERK) [9–11]. MLK3 (aka MAP3K11) is the most widely expressed MLK family member [9–11] and is known to be expressed in neurons [12], dendritic cells [13, 14], and many other cell types. At the cellular level, MLK3 is activated by cellular/metabolic stress, including reactive oxygen species, ceramide and TNF-α [15, 16]. At the molecular level, it is activated by Cdc42 and Rac, which interact with MLK3, and can cause it to dimerize via a leucine zipper interface, resulting in autophosphorylation at Thr277 and Ser281 within the protein activation loop, and enzyme activation [17, 18]. Our research group at the University of Rochester Medical Center has shown that HIV-1 Tat also leads to phosphorylation at these same residues in primary rat neurons [19] and that HIV-1 Tat also leads to activation of glycogen synthase kinase (GSK)-3β in neurons [20, 21]. Interestingly, these seemingly disparate events may be causally connected, since recent findings have shown that MLK3 can be activated as a result of direct phosphorylation by GSK-3β [22]. Endogenous inhibitors of MLK3 include the pro-survival protein kinase, Akt [23, 24]. Finally, in addition to its conventional kinase activity, MLK3 also possesses noncatalytic functions that contribute to activation of the Raf/ERK pathway and induction of cell proliferation; these effects are negatively regulated by the tumor suppressor merlin [25].

Role of MLK3 in neurodegenerative diseases

MLK3 has been implicated in neuronal apoptosis leading to neurodegenerative diseases [19, 22, 26, 27]. In the context of Parkinson’s disease (PD), the first-generation MLK3 inhibitor, CEP-1347 (Fig. 2), has been shown to prevent the induction of neuronal cell death, motor deficits and neuronal degeneration in the MPTP model of Parkinsonism [28–31]. CEP-1347-mediated neuroprotection has also been demonstrated in an *in vitro* model for PD, using methamphetamine-exposed human mesencephalic-derived neurons[31]. This has led to the use of CEP-1347 in a large Phase II study in patients with PD (see below).

Activation of MLK3 and downstream kinases (JNK, p38) has also been implicated in the pathogenesis of several other neurodegenerative diseases [11], including HIV-associated dementia [19, 32], Alzheimer's disease (AD) [33, 34], and ischemic injury/stroke [35–40]. MLK3's role in ischemic injury may extend to other tissues, such as heart, where a first-generation MLK3 inhibitor (CEP11004) has been shown to reduce myocardial cell death and restore post-ischemic contractile function [41].

In addition, MLK3 has been implicated as playing a causal role in peripheral neuronal degeneration, including the development of HIV-associated peripheral neuropathy, which can be induced both by soluble HIV-1 gene products and also by the antiviral drugs used to treat HIV-1 [42, 43]. Finally, the first generation MLK3 inhibitor, CEP-1347, has been shown to prevent the death of vestibular and cochlear hair cells in models for ototoxicity caused by exposure to aminoglycoside antibiotics [44–46].

Role of MLK3 in inflammation and immunity

One contributing factor to the neuroprotective efficacy of MLK3 blockade is the fact that MLK3 activation plays an essential role in the activation of microglia and astrocytes, and their subsequent release of proinflammatory cytokines [47, 48]. Thus, MLK3 likely plays an important role in inflammation and immunity. Consistent with this, MLK3 is expressed in dendritic cells [13, 14] and regulates CD3/CD28-mediated signaling events in T cells [49].

Development and clinical evaluation of first-generation MLK3 inhibitors

Cephalon's CEP-1347 (Fig. 2) is the first and to date, the only inhibitor showing significant MLK3 activity that has been tested in human subjects. The compound is not completely specific for MLK3, and there is no published data that quantify its ability to penetrate the CNS. It is a large molecular weight compound (MW = 615) with high polar surface area (95 square angstroms), properties that are known to limit CNS penetration. CEP-1347 is an ethylthiomethyl analog of K-252a, a natural product indolocarbazole isolated from the bacterium *Nocardiopsis* species [12]. CEP-1347 demonstrated neuroprotective activity in preclinical models for PD, which were sufficiently compelling to initiate early Phase 1 studies to demonstrate the safety and tolerability of CEP-1347 in patients suffering from PD [50], followed by a larger blinded, placebo-controlled trial of efficacy in patients with early untreated PD (PRECEPT study) [51].

The MLK3 inhibitor CEP-1347 was safe and well tolerated in human subjects [50] but was an ineffective treatment in subjects with early PD [52]. We believe it is likely that early symptomatic PD patients may already have an advancing underlying disease that is not readily amenable to therapeutic intervention [52]. Also, failure of the PRECEPT trial may reflect dosage considerations related to the bell-shaped efficacy curve for CEP-1347 and/or failure to maintain adequate therapeutic levels of CEP-1347 within the CNS. It is also possible that an additional reason for failure of the PRECEPT trial may be related to the fact that MLK3 inhibition has both a cell survival-promoting effect and an inhibitory effect on neuroinflammation. These represent strong synergistic neuroprotective activities in the context of preclinical models for PD, as well as in human neurodegenerative diseases such as neuroAIDS and AD, which are characterized by a combination of neuronal damage/

neuronal loss plus a profound neuroinflammatory reaction associated with the release of neurotoxic inflammatory mediators. In neuroAIDS and AD, the synapse is likely to be the primary locus of dysfunction that is the substrate for neurologic disease and thus MLK3 inhibition may be a particularly valuable strategy for therapeutic intervention. In the case of early PD, neuroinflammation does not appear to be a major component of nigrostriatal degeneration. Thus, this may not have been an ideal target population for evaluation of the neuroprotective efficacy of CEP-1347.

MLK3 inhibitors and microglial inflammation

Researchers at Lundbeck A/S demonstrated that CEP-1347 reduced cytokine production in human and murine microglial cell cultures, and in monocyte/macrophage-derived cell lines that were stimulated with various endotoxins or the plaque forming peptide Aβ1-40. CEP-1347 inhibited brain TNF-α production induced by intracerebroventricular injection of LPS (lipopolysaccharide) in mice. The authors postulated that MLKs may function as significant modulators of microglial inflammation and demonstrated anti-inflammatory potential for an MLK3 inhibitor [48].

Efficacy of CEP-1347 in murine HIVE model

In our own experiments, HIV infected monocyte derived macrophages were stereotactically injected into the basal ganglia of CB17 severe combined immunodeficient mice (murine HIVE model) [53]. Daily intraperitoneal injections of CEP-1347 (up to 15 mg/kg) produced a dose dependent reduction in microgliosis measured by post mortem staining with Iba-1, suggesting the value of further evaluation of the neuroprotective properties of MLK3 inhibitors with better blood brain barrier penetration [54].

Development of novel MLK3 inhibitors

The human clinical experience with CEP-1347 demonstrated that MLK3 blockade is safe and well tolerated [50]. This is consistent with the fact that MLK3 knockout mice have no discernable phenotype, other than a selective reduction in tumor necrosis factor (TNF)-stimulated JNK activation [55]. We believe that there are compelling reasons to develop new, second-generation inhibitors of MLK3. CEP-1347 is based on the molecular scaffold of a staurosporine analog (K252a), which imposes a concern with respect to kinase selectivity (staurosporine being a very broadly active kinase inhibitor [56]). Moreover, CEP-1347 has complex biological effects that include not only inhibition of MLK3 [12] but also unrelated and/or off-target effects such as activation of Akt and Erk [57]. Finally, CEP-1347 exhibits a bell-shaped efficacy curve *in vivo* and is effective only over a relatively narrow dose concentration [28, 29]. For example, a dose of 0.3 mg/kg/day was highly effective in attenuating the loss of substantia nigra TH immunoreactive neurons after MPTP lesion in mice, but a dose of 3 mg/kg/day was markedly less effective and a dose of 0.03 mg/kg/day was completely ineffective [28]. Similar findings have been reported in a nonhuman primate model for PD [29]. In light of these considerations, we have developed improved second-generation MLK3 inhibitors with novel molecular structures distinct from CEP-1347 and proven CNS penetrance in pharmacokinetic studies. Thus, our initial goal was to derive inhibitors with enhanced specificity, reduced off-target effects, and a more

favorable window of efficacy compared to CEP-1347. We achieved this goal after a focused approach to design drug-like compounds with favorable CNS profiles and nanomolar potency for MLK3, recently reporting on a small molecule MLK3 inhibitor, URMC-099, as a potential first-in-class adjunctive therapy for HAND [58, 59]. Further, in our goal to establish compatibility of URMC-099 with current cART, we serendipitously discovered that URMC-099 boosts antiviral activities of long acting antiretroviral therapy. Specifically, URMC-099 potentiates antiretroviral actions of nanoformulated ritonavir-boosted atazanavir (nanoATV/r). This drug combination led to a marked reduction of residual HIV-1 infection. URMC-099 facilitated nanoATV/r therapeutic effects by affecting the expression of the Rab family proteins that regulate endosomal vesicle trafficking, augmenting interactions between nanoATV/r and the viral life cycle. This combination of a MLK3 inhibitor with anti-inflammatory and neuroprotective properties with nanoART has led to the concept to treat persistent HIV-1 infection. The observation supports the idea that MLK3 inhibition uniquely targets sites of viral maturation with the ability to synergistically act with ART to prevent the viral life cycle from successfully achieving productive infection without accompanying cell death or inflammation.

Conclusion

Future strategies to achieve eradication of HIV infection in its CNS sanctuary must both target the virus and its life cycle and achieve resolution of associated neuroinflammation. We posit that one way this may be achieved is by improved targeting of ART with nanoformulations and accompanying ART treatment with disease modifying adjunctive therapies.

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Abbreviations

AD	Alzheimer’s Disease
CNS	central nervous system
JNK	c-Jun N-terminal kinase
CPE	CNS penetration score
cART	combination ART
ERK	extracellular signal-regulated kinase
GSK	glycogen synthase kinase
HAND	HIV-associated neurocognitive disorders

MLK3	mixed lineage kinase 3 inhibitor
MAPK	mitogen activated protein kinase
MKKKs	mitogen activated protein kinase kinase kinases
nanoART	nanoformulated antiretroviral therapy
nanoATV/r	nanoformulated ritonavir-boosted atazanavir
PD	Parkinson's Disease

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▪ of special interest

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Key points

- Anti-inflammatory events need be eliminated in order to affect the secondary consequences of CNS viral infections
- Chemical eradication of HIV infection in its CNS reservoirs could be achieved by combined approaches that improve delivery of ART to its sites of action
- MLK3 inhibitors affect ART efficacy by affect intracellular trafficking of nanoparticles

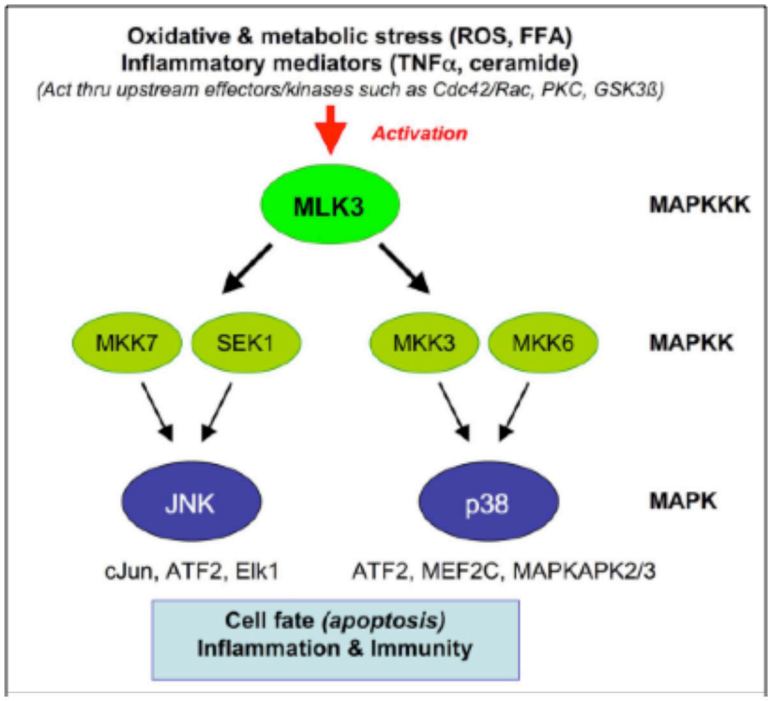

Figure 1.
Mixed lineage kinase 3 regulates the JNK and p38 pathways.

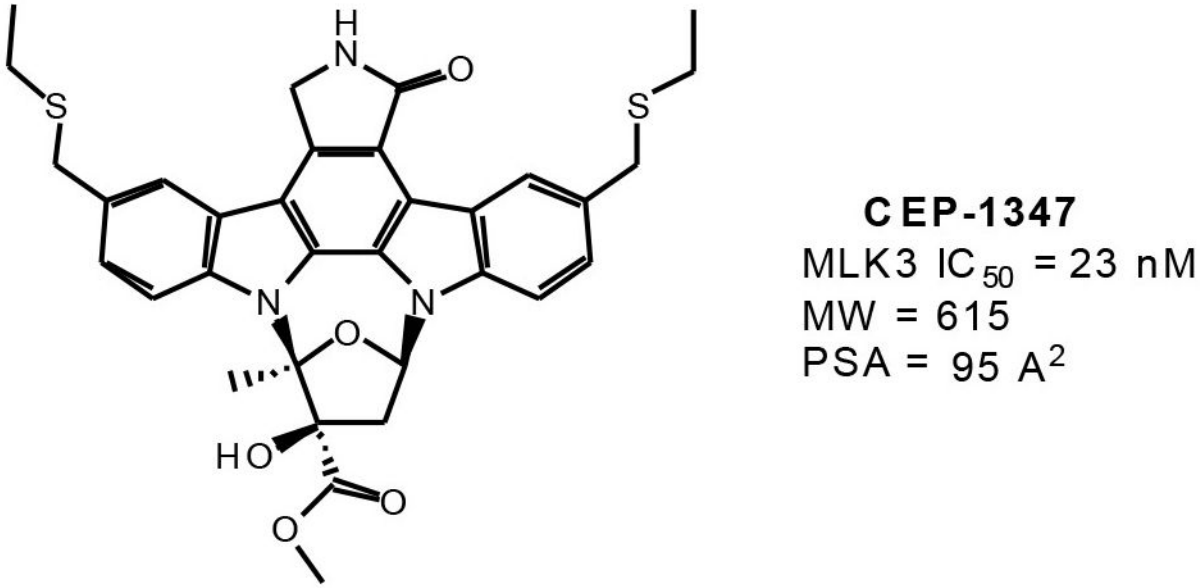

Figure 2.
Chemical structure, MLK31C50, and physical properties of CEP-1347.