Increased Activity of Matrix Metalloproteinase-2 in Human Glial and Neuronal Cell Lines Treated with HIV-1 gp41 Peptides

Young Hae Chong,* Ju Young Seoh, and Hae Kyung Park

Department of Microbiology, College of Medicine, Division of Molecular Biology and Neuroscience, Medical Research Center, Ewha Womans University, 911-1, Mok-6-dong, Yangcheonku, Seoul, Korea, 158-056

Received January 2, 1998; Accepted March 13, 1998

Abstract

Part of the neurodegenerative cascade in AIDS dementia may involve overexpression of matrix metalloproteinases (MMPs). Here, we examined the possible effect of HIV-1 gp41, which has been shown as a key determinant associated with pathogenesis of AIDS dementia, on the activity of MMPs using human neuronal and glial cell lines. Zymographic analysis revealed that treatment with the gp41 peptide (aa 583-599) for 24 h markedly elevated the activity of MMP with Mr 66 kDa in the cultured media of glioblastoma cell line T98G in a concentration-dependent manner as well as of neuroblastoma cell line SK-N-SH despite of lower magnitude of the acticity. In contrast, the immediately adjacent gp41 peptide (aa 598-613) as well as the reverse peptide (aa 598-583) had a little effect. Recombinant gp41 protein containing extracellular domain also elicited a similar effect, although with a lesser extent. This 66 kDa MMP was confirmed as gelatinase A (MMP-2) based on the results of its activity dependent on Ca$^{2+}$ and inhibited in the presence of 1,10-phenanthroline or EDTA, as well as its specific immunoreactivity on the Western blot. N-acetyl cysteine (NAC) downregulated this gp41 peptide-induced MMP-2 activity in T98G. The soluble form of amyloid precursor protein (sAPP), which is synthesized in the Escherichia coli system, also inhibited the MMP-2 activity in vitro. Taken together, these results implicate that high production of HIV-1 gp41 or its metabolites containing aa 583–599 within central nervous system (CNS) could result in the increased activity of MMP-2 and that the extracellular deficiency of reducing agent or decreased level of sAPP within CNS could exacerbate this gp41-induced MMP-2 activity.

Index Entries: AIDS dementia; HIV-1 gp41 peptides; matrix metalloproteinase-2 (MMP-2); N-acetyl cysteine (NAC); secreted form of amyloid precursor protein (sAPP).

*Author to whom all correspondence and reprint requests should be addressed.
Introduction

The mechanism(s) responsible for human immunodeficiency virus 1 (HIV-1) invasion of the central nervous system (CNS) associated with the generation of AIDS dementia is unclear. Indirect mechanisms that involve viral gene products, and cytokine dysregulation and other cellular factors released by HIV-1-infected brain macrophages and microglia as well as reactive astrocytes (Brew et al., 1995; Nottet et al., 1995; Glass and Johnson, 1996) are implicated in the severity of neurological impairment that occurs in AIDS dementia. Earlier data reported that the HIV-1 coat protein gp120, shed from the virus, could contribute to neuronal degeneration by excitotoxic mechanisms (Toggas et al., 1994) or by cytokine induction (Yeung et al., 1995). However, the extent of dendritic and neuronal damage in HIV encephalitis may be more closely correlated to the amount of HIV-1 transmembrane (TM) protein gp41 in the brain (Masliah et al., 1992). Furthermore, recent data strongly implicated a possible association between increased expression of gp41 in HIV-1-infected brains and the cognitive dysfunction in AIDS-associated dementia (Adamson et al., 1996). These observations suggest that gp41 could be a key determinant in neurodegenerative cascade, ultimately leading to neuronal damage and dementia.

Several potential cytopathic mechanisms of the HIV-1 gp41 protein include syncytium formation by the hydrophobic fusion domain (Bergeron et al., 1992), inhibition of lymphoproliferation by the immunosuppressive peptide (Ruegg and Strand, 1991; Denner et al., 1994). These multiple cytopathic effects have been associated with peptide sequences near the N-terminal ectodomain of TM gp41 protein. In addition to these components in extracellular domain, potentially cytopathic regions have been located in the C-terminal cytoplasmic tail of gp41 (Miller et al., 1994). Despite such extensive studies for multiple cytopathic immune-modifying properties of gp41, potential roles of these gp41-derived peptides within HIV-infected brains have not been extensively explored. Thus, unraveling the biological function of HIV-1 gp41 within CNS remains critical to understanding the neuropathogenic mechanisms of AIDS-associated dementia.

Recent in vitro studies suggest that dysregulation of the synthesis and the release of matrix metalloproteinases (MMPs) by HIV infection may contribute to viral dissemination and tissue damage as seen in patients during the progression of AIDS (Weeks et al., 1993; Chapel et al., 1994; Kalebic et al., 1994) or decreased antiviral immune state during disease progression (Mariani et al., 1995; Sieg et al., 1997). The expression of MMPs is increased in HIV-infected lymphocytes (Weeks et al., 1993) and monocyte-derived macrophages (Chapels et al., 1994). MMPs, enzymes produced by neurons and glia, were also found to be elevated in cerebrospinal fluid (CSF) of subjects with a variety of inflammatory CNS neurologic disorders as well as in hippocampal tissue of Alzheimer's disease (AD) (Backstrom et al., 1992; Gijbels et al., 1992). However, little is known about the levels, functions, and viral or cellular factors that are responsible for the modulation of MMPs in HIV-1 infected brains. Thus, it will be of particular interest to examine the viral or cellular factors that could modulate the activity of MMPs in glia and neurons with regard to investigating possible pathogenic mechanisms involved in HIV-1-associated dementia and developing therapeutic strategies in the control of HIV pathogenesis.

In this study, we determined whether HIV-1-derived gp41 peptides could exacerbate the activity of MMPs in cultured human neuronal and glial cell lines, such as a T98G glioblastoma cell, which has been proven to be useful model for astrocyte function in vivo (Fontana et al., 1984), and an SK-N-SH neuroblastoma cell (Ogino et al., 1992). In addition, we examined the effect of reducing agents, such as N-acetyl cysteine (NAC) and glutathione as well as anti-inflammatory agents, including dexamethasone and indomethacin, in order to identify agents that could modulate MMP activity mediated by gp41 peptide treatment. The recombinant soluble form of amyloid precursor protein (sAPP), which has been demonstrated as a trophic and protective factor within CNS (Mattson et al., 1993; Furukawa et al., 1996), was synthesized using the Escherichia coli expression system and tested its effect on MMP activity in vitro.