Curcumin improves spatial memory impairment induced by human immunodeficiency virus type 1 glycoprotein 120 V3 loop peptide in rats

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Aims: Human immunodeficiency virus-1 (HIV-1)-associated dementia (HAD) is a significant consequence of HIV infection. Although highly active antiretroviral therapy (HAART) has dramatically decreased HIV-1 load in acquired immune deficiency syndrome (AIDS) patients, HAART does not completely protect against the development of HAD, therefore novel strategies for the prevention and treatment are urgently needed. In this study, we chose curcumin which has a neuroprotective role and tested the effect against neuron damage induced by HIV-1gp120 V3 loop peptide.

Main methods: Rats were given 150 ng gp120 V3 peptide by intracerebroventricular (ICV) infusion for 3 days to establish the cognitive dysfunction model. After recovery from the surgery, the rats in treatment groups were given curcumin by intragastric infusion for 2 weeks. Subsequently, we used the Morris water maze test, long-term potentiation (LTP) recording, biochemical measurement of oxidative damage, Nissl staining, and BDNF immunostaining to evaluate the neuropathological changes and the effect of curcumin on rats.

Key findings: Our results documented that the gp120 V3 peptide induced impairment of spatial learning and memory, inhibited LTP in the CA1 region of the hippocampus, and mediated oxidative stress and neuronal injury. These impairments were ameliorated by intragastric infusion of curcumin.

Significance: These results suggested that dietary supplementation of curcumin may be a potential therapeutic strategy for the treatment and/or prevention of HAD.

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Introduction

Over the past two decades, acquired immune deficiency syndrome (AIDS) caused by human immunodeficiency virus-1 (HIV-1) has emerged as one of the most visible and important health concerns worldwide. HIV-1 enters the central nervous system (CNS) at an early stage (Chakrabarti et al. 1991; Davis et al. 1992), which causes significant damage in the nervous system during the disease course of AIDS. That eventually leads to a variety of progressive neurological disorders, including HIV-1-associated dementia (HAD). HAD is characterized by deterioration of cognitive and motor functions and behavioral changes (Navia et al. 1986; Price et al. 1988).

Even though HAD incidence has decreased to less than 10% of AIDS patients with the use of highly active antiretroviral therapy (HAART) (Dore et al. 1999; Sacktor et al. 2001), HAD still is a significant complication of advanced HIV-1 disease. Extensive data suggest that the cognitive impairments seen in AIDS patients are associated with the brain infiltration of HIV-1 infected mononuclear phagocytes (MP; perivascular brain macrophages and microglia) (Glass et al. 1995) and the resulting release of soluble viral (e.g. HIV-1gp120) and cellular factors (such as proinflammatory cytokines). These soluble factors induce neuronal apoptosis (Adle-Biassette et al. 1995; Gelbard et al. 1995; Petito and Roberts 1995), reduction in synaptic and dendritic densities (Everall et al. 1999; Fox et al. 1997), and selective neuronal loss (Fox et al. 1997; Maslia et al. 1992). However, the molecular and cellular pathogenesis of HAD is not understood. Therefore, understanding of the underlying mechanisms by which HIV-1 causes HAD and an exploration of a new approach for the treatment of HAD is imperative (Bouwman et al. 1998; Langford et al. 2003; Sacktor et al. 2002).

Curcumin, a principal curcuminoid of the Indian curry spice turmeric, has been widely used for centuries in indigenous medicine for the treatment of a variety of inflammatory conditions and diseases. It has a wide range of pharmacological properties, including anti-
inflammatory (Satoskar et al. 1986; Sandur et al. 2007), anti-cancer (Kuttan et al. 1985; Anand et al. 2008), anti-oxidant (Manikandan et al. 2004; Toda et al. 1985), wound healing (Sidhu et al. 1999), and antimicrobial effects (Negi et al. 1999). Dietary supplementation with curcumin is beneficial in neurodegenerative disorders such as Alzheimer’s disease (Calabrese et al. 2003; Yang et al. 2005). In a focal cerebral ischemia model of rats, curcumin offers significant neuron protection through the inhibition of lipid peroxidation, an increase in endogenous antioxidant defense enzymes, and a reduction in peroxynitrite formation (Thiyagarajan and Sharma 2004). Therefore, we hypothesize that curcumin may be able to treat or prevent HAD by a reduction in HIV-induced neurotoxicity. To test this hypothesis, we evaluated the neuron protective effects of curcumin in rats with cognitive deficits induced by the intracerebroventricular (ICV) injection of HIV-1 gp120 V3 loop peptide.

Materials and methods

Chemical and biological reagents

HIV-1MN V3 loop peptide (V3, 23-mer, cyclic, AATGLS IAIGAGPG AAPVTIGLIG-NH2) was purchased from Sigma (St. Louis, MO). Curcumin was obtained from Fluka (Völklingen, Switzerland). Reagent kits for superoxide dismutase, malondialdehyde, glutathione, hydroxide free radical, and biuret protein were purchased from Jancheng Bioengineering Research Institute (Nanjing, China). Brain-derived neurotrophic factor (BDNF) monoclonal antibody was obtained from Boster Biotechnique L.C. (Wuhan, China).

Animals

Ninety-five Sprague–Dawley rats of both sexes (three- to five-weeks-old with 80–100 g body weight) were purchased from Guangdong Experimental Animal Center (animal grade: KwangTung province 2006A015). Five additional rats were included to compensate for surgical death. The rats were housed at a constant temperature (22 °C) and relative humidity (50%) under a regular light–dark cycle (light on at 7 AM and off at 5 PM) with free access to food and water. Three days after adaptation, the rats were randomly assigned to six different groups (n = 15 for each group): 1) control, 2) sham, 3) V3, 4) V3 + 50 mg/kg curcumin, 5) V3 + 100 mg/kg curcumin, and 6) V3 + 200 mg/kg curcumin. Curcumin was dissolved in 0.5% sodium carboxymethyl cellulose solution with various final concentrations (5 mg/ml, 10 mg/ml, and 20 mg/ml). The rats in the control treatment groups (groups 4, 5, and 6) received curcumin daily via intragastric infusion (1 ml/100 g body weight). The rats in the control group did not receive any treatment, and the rats in the sham group were intrastratically infused with the same volume of distilled water as the rats in the curcumin treatment groups did. The animal protocol was strictly reviewed by the Institutional Animal Care Committee of Jinan University.

Stereotoxic surgery and ICV injections

The rats were anesthetized with sodium pentobarbital (50 mg/kg, ip) and placed in a stereotoxic apparatus. A burr hole was drilled 1 mm posterior to the bregma and 1.5 mm lateral to the midline, and a 22-gauge plastic cannula was placed 4 mm below the skull surface. The guide cannula was secured to the skull with glue and dental cement. The extracranial tip of the plastic cannula was then sealed via an open flame to ensure that the cannula remained patent. To minimize the stress and infection during the experiment, the rats were given benzylpenicillin sodium (300,000 IU/kg, im) for 3 days. By the end of surgery, four rats were excluded because of surgical accident. Four to five days after the surgery, the rats in the group 3, 4, 5, and 6 were given 50 mg V3 loop peptide (dissolved in 5 μl artificial cerebrospinal fluid [ACSF]) daily for 3 consecutive days. The infusion was achieved by the connection to the plastic cannula with a micro-syringe (Hangzhou, China) at a constant rate of 1 μl/min. The micro-syringe was allowed to stay for another 5 min after the termination of the infusion to avoid spillage from the guide cannula. The rats in the sham group received ACSF (5 μl/day) for 3 consecutive days, while the rats in the control group did not undergo any surgical operation (more operation details please see Table 1).

Morris water maze (MWM) testing

MWM was used to test spatial memory acquisition and retention in the rats (Morris et al. 1982; Sutherland et al. 1982). The water maze consisted of a black circular pool with a diameter of 130 cm and a height of 50 cm, filled with 24 ± 1 °C water to a depth of 20 cm. The maze was divided into four equal quadrants and release points were designated in each quadrant as north, east, south, and west. A hidden circular platform (12 cm in diameter), made of plexiglass, was placed in the center of the southwest quadrant and submerged 2 cm beneath the water surface. White biodegradable fresh milk was added to the water to prevent the rats from seeing the platform. We used a four-trials-per-day per rat, 5-day MWM training protocol, a good discriminative test for the effect of exercise on learning and memory (Vaynman et al. 2004). The rats were placed in the maze facing the wall from one of the equally spaced start locations, which were randomly altered every trial. Spatial reference cues around the pool were maintained constantly during the MWM training and probe trials. Each trial lasted until the rat found the platform or for a maximum duration of 60 s (seconds). If the rat failed to find the platform, it was gently placed on the platform. At the end of each trial, the rat was allowed to rest on the platform for 20 s. To assess spatial memory retention, a probe trial was performed the day after the last training day. At this time, the platform was removed from the pool, while all other factors remained the same. Meanwhile, the efficient retention time and the efficient crossing platform frequency were measured for 60 s, and the rats’ swim paths were semi-automatically recorded by a video tracking system (Spontaneous Motor Activity Recording).

All equipments and materials mentioned above were used to determine whether V3 loop peptide has an effect on spatial learning and memory in rats, and next to evaluate further the influence of V3 loop peptide on motor behavior in rats we used visible platform. After spatial learning and memory training, another 36 rats were selected (divided into 6 groups each) for targeted swimming test using a visible platform, which stayed at the same location during 5 consecutive days. Each rat in the different groups performed four trials per day and started at different locations, which followed a north–east–south–west circle. Meanwhile, swimming time was calculated for finding the visible platform.

Hippocampal slices and electrophysiology

Rat hippocampal brain slices were prepared as previously described (Xiong et al. 1996). Briefly, the rats were anesthetized and decapitated, and their brains quickly removed from the cranial cavity. The brains were placed into an ice-cold (4 °C) oxygenated ACSF. The hippocampi were dissected and 400 μm transverse hippocampal slices were cut using a vibration tissue slicer (WPI, USA). The slices were kept in a humidified and oxygenated chamber at room temperature for at least 1 h before being transferred into a recording chamber. In

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
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<th>V3 + curcumin treatment</th>
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<td>ICV injection</td>
<td>No</td>
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<td>V3</td>
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<td>ING injection</td>
<td>No</td>
<td>No</td>
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ICV injection: intracerebroventricular injection, ING injection: intragastric injection.
the recording chamber, single hippocampal slices were fully submerged and continuously perfused with ACSF at a constant flow rate of 2 ml/min with the use of a peristaltic pump (WPI, USA). The ACSF contained 124.0 mM NaCl, 3.0 mM KCl, 2.0 mM CaCl$_2$, 2.0 mM MgCl$_2$, 1.25 mM NaH$_2$PO$_4$, 26.0 mM NaHCO$_3$, and 10.0 mM glucose. ACSF was equilibrated with 95% O$_2$ and 5% CO$_2$ and had a pH of 7.4 to generate approximately 30 mmol/mg protein.

**Staining and immunohistochemistry**

To investigate the histopathological changes, a subgroup of rats were deeply anesthetized with sodium pentobarbital and subsequently perfused via the heart with 200 ml of physiological saline (containing 0.1% heparin), followed by 500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer solution (PBS, pH 7.4). The brains were then removed, deeply anesthetized with sodium pentobarbital and subsequently fixed in 4% paraformaldehyde solution for 24 h at 4 °C, and embedded with paraffin. Subsequently, 4 μm coronal paraffin sections were cut, mounted onto gelatin-coated slides, and stored at 60 °C for 3 h prior to staining preparation. The deparaffinization protocol was used as described (Zhou and Baudry 2006). Nissl staining and BDNF immunostaining were carried out following the instruction manual.

**Statistical analysis**

Data are expressed as means ± SEM. MWM data were analyzed using repeated measure analysis with SPSS 13.0, and statistical
analysis was carried out using one-way ANOVA, followed by the Student’s Newman Keuls test. Other statistical analyses were made via two-tailed \( t \)-tests or one-way ANOVA. Differences were considered significant if \( P < 0.05 \).

**Results**

Effect of curcumin on gp120 V3 loop peptide-induced spatial learning and memory deficits

To investigate the effect of curcumin on gp120 V3 loop peptide-induced impairment of spatial learning and memory, we used Morris water maze (MWM) protocol, an established method for discerning learning differences (Vaynman et al. 2004). First, all rats were trained on the MWM task for 1 day in order to acclimate them to water maze performance, then all rats were trained for five consecutive days as part of the formal experiment.

On the first day, rats in the control group and the sham group spent less time to find the target quadrant compared with the other four groups: the V3 group, the V3+50 mg/kg curcumin group, the V3+100 mg/kg curcumin group, and the V3+200 mg/kg curcumin group (Fig. 1). We noted that the rats in the V3 group were uncoordinated and required a longer time to find the hidden platform than the rats in the control group and the sham group during five training days, suggesting that V3 impaired spatial learning ability. Specifically, the escape latencies of the V3+50 mg/kg curcumin group (11.41±2.97 s) were significantly shorter than the V3+100 mg/kg and the V3+200 mg/kg curcumin groups (16.47±6.50 s, 18.22±9.16 s) at day 5 (\( F = 41.53, P < 0.01 \)), indicating that application of 50 mg/kg curcumin reversed the impairment of spatial learning ability significantly (Fig. 1B).

After the rats had learned the hidden platform location, the platform was removed from the pool to test memory retention. The rats of the control and sham groups spent a longer time in the target quadrant searching for the platform than the other four groups (V3, V3+50 mg/kg curcumin, V3+100 mg/kg curcumin, and V3+200 mg/kg curcumin),

![Effect of curcumin on targeted swimming test with behavior impairment induced by gp120 V3 loop peptide in MWM task (A and B).](image)

Fig. 3. Effect of curcumin on targeted swimming test with behavior impairment induced by gp120 V3 loop peptide in MWM task (A and B). *P < 0.05 vs. the sham group, the number of rats was 6 within each group. Six groups: 1, control; 2, sham; 3, V3; 4, V3+50 mg/kg curcumin; 5, V3+100 mg/kg curcumin; 6, V3+200 mg/kg curcumin.
suggesting that rats in the control and the sham groups could recall the location of the hidden platform (Fig. 2). The rats of the V3 group spent significantly less time in the target quadrant (3.92 ± 1.04 s, n = 15) and less frequently crossed into the target quadrant (3.13 ± 1.23 /min) compared with the control group (8.17 ± 0.75 /min, P < 0.05), indicating that V3 caused spatial memory deficits. However, the rats of the V3 + 50 mg/kg curcumin group spent significantly more time in the target quadrant (7.25 ± 0.98 s) and crossed the target quadrant more frequently (6.25 ± 1.04 /min, Fig. 2) than the V3 + 100 mg/kg and V3 + 200 mg/kg curcumin treatment groups, indicating that 50 mg/kg curcumin can significantly improve spatial memory ability.

To determine whether the gp120 V3 loop peptide has effects on motor behavior, a targeted swimming test using a visible platform was conducted. At the beginning of the test, the rats in the control group and the sham group found the platform faster than the rats of the V3 group (Fig. 3), and this difference was maintained at all five testing intervals and was statistically significant at day 1, day 3, and day 5 (Fig. 3B, P < 0.05), suggesting that V3 caused the dysfunction of motor behavior. The rats in the V3 + 50 mg/kg curcumin group, the V3 + 100 mg/kg curcumin group, and the V3 + 200 mg/kg curcumin group spent less time finding the visible platform compared with the V3 group, but there was no significant difference between these three groups. Fig. 3 shows that the swimming time of the three curcumin treatment groups relatively decreased. However, application of curcumin did not significantly improve motor behavior. These results showed that V3 indeed caused the impairment of spatial learning and memory and the dysfunction of motor behavior, and curcumin improved spatial learning and memory statistically.

**Fig. 4.** LTP in the CA1 region of rat hippocampal slices in different groups. A. EPSP initial slope of rats in control group, the V3 group and the V3 + 50 mg/kg curcumin group. B shows the bar graph of LTP initial slope in different groups. *P < 0.05 vs. control group, †P < 0.05 vs. the V3 group, ‡P < 0.01 vs. V3 + 50 mg/kg curcumin group. The number of rats is 5 in each group. Six groups: 1, control; 2, sham; 3, V3; 4, V3 + 50 mg/kg curcumin; 5, V3 + 100 mg/kg curcumin; 6, V3 + 200 mg/kg curcumin.

**Fig. 5.** Effect of curcumin anti-oxidative effect with memory impairment induced by gp120 V3 loop peptide. Activity tests of MDA (A), OH· (B), SOD (C) and GSH (D) in six groups. MDA, malondialdehyde; SOD, superoxide dismutase; GSH, glutathione. (ANOVA, *P < 0.05 vs. the control group, †P < 0.05 vs. the V3 group, ‡P < 0.01 vs V3 + 50 mg/kg curcumin group (n = 6 in each group). Six groups: 1, control; 2, sham; 3, V3; 4, V3 + 50 mg/kg curcumin; 5, V3 + 100 mg/kg curcumin; 6, V3 + 200 mg/kg curcumin.
Effect of curcumin on gp120 V3 loop peptide-induced decrease of LTP

Our previous experiment documented that incubation of HIV-1 gp120 inhibited long-term potentiation (LTP) in the rat hippocampal slices in vitro. To examine whether gp120 V3 loop peptide injected by ICV had an effect on LTP, we tested the initial slope of LTP. The rats in the V3 group had significantly less magnitude of LTP than the rats in the control group (Fig. 4), suggesting that V3 inhibited LTP in the CA1 region of the hippocampus. As anticipated, there was no significant difference in the magnitude of LTP between the control group and the sham group, indicating that surgery had no effect on LTP recording. The average magnitudes (% of basal level) of LTP in the V3 + 50 mg/kg curcumin group (136.91 ± 9.28%), in the V3 + 100 mg/kg curcumin group (109.76 ± 7.6%), and in the V3 + 200 mg/kg curcumin group (110.57 ± 8.09%) were much larger than in the V3 group (P < 0.05), suggesting that the inhibition of LTP was reversed by curcumin treatment. Furthermore, application of 50 mg/kg curcumin had a significant effect on reversing the inhibition of LTP caused by gp120 V3 loop peptide (P < 0.01). These combined results showed that gp120 V3 loop peptide caused the inhibition of LTP, which was ameliorated by the application of curcumin. Moreover, the V3 + 50 mg/kg curcumin group had a significantly larger LTP magnitude than the other two curcumin treatment groups.

Effect of curcumin on gp120 V3 loop peptide-induced oxidative damage

Considering that oxidative damage has been documented to participate in the process of neuronal injury in HAD (Turcan et al. 2003), we used oxidation indicators such as MDA and OH· to assess the effect of curcumin on gp120 induced oxidation damage. The V3 group had a significantly higher level of MDA and OH· than the other five groups (Fig. 5), indicating that gp120 V3 loop peptide caused the oxidation damage in the brain. Importantly, application of curcumin reduced the higher MDA and OH· concentration suggesting that curcumin reduced this oxidative damage. We also measured the concentrations of anti-oxidative indicators such as SOD and GSH. The V3 group had a significantly lower concentration of SOD and GSH than the control and sham groups. Curcumin treatment increased SOD and GSH levels. Furthermore, the V3 + 50 mg/kg curcumin group also had higher SOD and GSH levels than the other two curcumin treatment groups, but this was not statistically significant. These combined results indicate that curcumin protects against gp120 V3 loop peptide-induced oxidative damage.

Effect of curcumin on gp120 V3 loop peptide-induced hippocampal structural abnormalities

We also used Nissl staining to monitor the hippocampal structural abnormalities. Nissl staining is an accepted method to detect the structural abnormalities of the hippocampi (Fig. 6). The control group and the sham group showed intact and well-arranged pyramidal neurons in the CA1 region (Fig. 6A and B). In contrast, the V3 group showed reduced staining of pyramidal neurons, which were significantly swollen and arranged abnormally (Fig. 6C). Quantitative analysis of neuron staining per unit area in the CA1 pyramidal cell layer was carried out, and neurons in the V3 group decreased (26.25 ± 1.28) significantly compared with the control group (34.75 ± 2.76) and the sham group (33.75 ± 1.75). This suggested that gp120 V3 loop peptide caused abnormal structure and neuron loss in the hippocampus, which has been linked to cognitive dysfunction. Application of curcumin diminished the loss and swelling of pyramidal neurons in the hippocampus, and all CA1 neurons in the V3 + 50 mg/kg curcumin group, the V3 + 100 mg/kg curcumin group, and the V3 + 200 mg/kg curcumin group were well-arranged compared with the V3 group (Fig. 6D, E, and F). Meanwhile, neurons in the V3 + 50 mg/kg curcumin group recovered their characteristic shape, and the number of CA1 neurons approached the normal number of the control group (31.75 ± 1.67).

Effect of curcumin on gp120 V3 loop peptide-induced BDNF immunoreactivity

All neurons in the CA1 and CA3 pyramidal cell layers were immunopositive for brain-derived neurotrophic factor (BDNF) (Fig. 7), and the BDNF immunoreactivity within the CA1 and CA3 pyramidal neurons was robust in the cytoplasm surrounding the
Additionally, the density of BDNF staining appeared uniform in each of these cell layers in the control and the sham groups (Fig. 7A & B). The density of BDNF stained in the V3 group was decreased in both the CA1 and CA3 pyramidal cell layers (Fig. 7C). To detect the differences between groups in specific regions, quantitative analysis of BDNF staining per unit area of different hippocampal cell layers was used. In the CA1 pyramidal cell layer, the V3 group had a significantly decreased number of BDNF staining cells than the control and the sham groups. Furthermore, the V3 + 50 mg/kg curcumin group had a significantly higher number of BDNF staining cells than the V3 + 100 mg/kg curcumin group and the V3 + 200 mg/kg curcumin group (Fig. 8A). Similarly, BDNF immunopositive cells in the CA3 pyramidal cell layer were consistently robust and regularly arranged in the control group and the sham group, while an absence of immunostaining in the V3 loop group was observed (Fig. 8C). The intensities of BDNF staining in three different curcumin treatment groups were significantly increased in comparison to the V3 group, while the V3 + 50 mg/kg curcumin group (52.38 ± 1.30) had significantly more BDNF staining cells than the V3 + 100 mg/kg curcumin group and the V3 + 200 mg/kg curcumin group. These

Fig. 7. BDNF immunoreactivity within the CA1 subfield (top panel) and the CA3 subfield (bottom panel). Panels A1 & A2 illustrate an example from the control group hippocampus, panels B1 & B2 show an example from the sham group hippocampus, panels C1 & C2 demonstrate an example from the V3 group hippocampus, panels D1 & D2 show the V3 + 50 mg/kg curcumin group and panels E1 & E2, F1 & F2 represent the V3 + 100 mg/kg curcumin group and the V3 + 200 mg/kg curcumin group. Note that: (i) all neurons in the CA1 and CA3 pyramidal cell layers were densely immunopositive; and (ii) BDNF expression appears diminished in CA1 & CA3 pyramidal cell layers of gp120 V3 loop peptide group. Magnification ×200.
analyses demonstrated that gp120 V3 loop peptide caused a down-regulation of BDNF expression in the hippocampus, which could be restored by the application of 50 mg/kg curcumin.

Discussion

HAD is characterized by a deterioration of cognitive functions, including memory (Masliah et al. 1997). The pathological basis of the cognitive impairment is unclear; however, several studies suggest that viral and immune products secreted from infected brain macrophages and microglia affect the onset and progression of the disease. One critical neurotoxin among these secretory products is HIV-1 envelope glycoprotein gp120. The toxic effect of gp120 on neurons has well been demonstrated (Dreyer et al. 1990; Kanmogne et al. 2002). Studies have shown that bilateral injection of 10–100 nM gp120 into the intermediate mesoderm of the chick forebrain causes amnesia, while several other studies shown that gp120 impairs memory retention in rodents (Glowa et al. 1992; Pugh et al. 2000; Sanchez-Alavez et al. 2000). Similarly, our previous experiment revealed that the V3 loop peptide produces an inhibition of LTP (Dong and Xiong 2006), a robust and long-lasting form of synaptic plasticity, which is widely considered a leading candidate for the cellular mechanism of mammalian learning and memory (Bliss and Collingridge 1993). In addition, the V3 loop peptide of gp120 was integral to many aspects of HIV-1 viral infectivity, suggesting that functional interaction must occur between the V3 loop region and the different coreceptors (Dettin et al. 2003). These studies indicated that gp120 V3 loop and synaptic plasticity were interrelated, and the studies of the mechanisms involved in this interface would open new avenues to understand better the cognitive dysfunction of HAD. Therefore, we used gp120 V3 loop peptide to induce cognition dysfunction and investigated the mechanism by which gp120 contributes to the pathologic process of HAD. Our study showed that ICV injecting the gp120 V3 loop peptide (150 ng of total concentration) into rats significantly reduced the learned acquisition of the MWM task, caused behavioral alteration, and diminished LTP. This notion was further supported by Nissl staining. The mechanisms of gp120 that cause neurotoxicity combine with multiple elements, including apoptosis (Kaul and Lipton 1999; Perfettini et al. 2005), oxidative damage (Price et al. 2005, 2006), interruption of the glutamate–glutamine cycle (Fernandes et al. 2007), and activation of various receptors (NMDA, CXCR4, CCR5, and so on) (Catani et al. 2000; Toggas et al. 1996). Regarding oxidative damage, the pathogenesis of HIV-1 infection has been implicated in increased levels of reactive oxygen species (ROS). Decreased levels of antioxidants and elevated levels of MDA (a reflection of increased levels of lipid peroxidation) have been reported in HIV-1 infected patients (Gil et al. 2003; Muller et al. 1996). These findings suggest that oxidative damage may contribute to the development of neuronal or synaptic dysfunction. Consistently, our results indicated that gp120 V3 loop peptide was associated with oxidative damage, contributing to cognitive deficits. This serves as further evidence to support the notion that free radical production and oxidative stress may play an important role in the pathogenesis of different neurodegenerative disorders, including HAD.

We further propose the use of drugs to reduce damage and promote cognition. However, a widely used HAD drug should have the following properties: an acceptable cost (less expensive than other anti-HIV drugs), minimal side effects, the capacity to cross the blood–brain barrier, and confirmed efficacy. Curcumin exhibited strong anti-oxidant activity comparable with vitamins C and E (Toda et al. 1985). It was shown to be a potent scavenger of a variety of reactive oxygen species, including superoxide anion radicals, hydroxyl radicals (Reddy and Lokesh 1992), and nitrogen dioxide radicals (Sreejayan and Rao 1997). Another critical characteristic of curcumin is its ability to cross the blood–brain barrier to affect direct neuroprotection (Yang et al. 2005). In our experiment, curcumin was intragastrically administered at 50 mg/kg, 100 mg/kg, and 200 mg/kg for two weeks, and was found to improve spatial learning and memory ability of rats caused by gp120 V3 loop peptide, reverse the inhibition of LTP, reduce oxidative damage in the post cortex and hippocampus of the gp120 V3 loop infected rats. Moreover, the application of 50 mg/kg curcumin had more efficient anti-oxidation damage to reverse the neuron damage compared with the other two dose curcumin treatment. It should be note that curcumin has been found to have ulcerogenic properties in some studies, causing gastric ulcerations in rats at doses of 100 mg/kg (Gupta et al. 1980). In addition, acute oral administration results in poor bioavailability, due to the rapid conversion to glucuronides (Kelloff et al. 1996), suggesting that small doses of curcumin may be necessary for a neuroprotective effect. Thus, we chose 50 mg/kg as a low dose, and gradually increased it for the other experimental groups. Another possible explanation for the differences observed between the three different curcumin treatment groups is that curcumin may have multiple mechanisms of action, specific to different doses and time courses, which may counterbalance each other at higher doses. One of the possible mechanistic roles of curcumin in gp120 V3 loop peptide-induced cognition deficit and
behavioral alteration was through its role of anti-oxidation, however, there was no statistical difference in the improvement of motor behavior in the three groups. Further motor behavior tests should be conducted to investigate the relative roles in neuronal protection.

During CNS development, BDNF is associated with neuronal plasticity. BDNF can modulate synaptic plasticity (Bolton et al. 2000; Kang and Schuman 1996), exhibits neuroprotective properties (Martinez-Serrano and Bjorklund 1996), and is required for normal learning in the Morris water maze (Mu et al. 1999). Furthermore, hippocampal BDNF seems necessary for the induction of LTP (Kovalchuk et al. 2002; Patterson et al. 1996). Some experimental evidence suggests gp120 toxicity can be reduced by BDNF, a naturally occurring peptide that has been shown to block neurotoxicity (Nosheny et al. 2005). Therefore, we propose that gp120 causes cognitive dysfunction by altering the expression of BDNF. Our results indicate that gp120 V3 loop peptide down-regulates the expression of BDNF in the hippocampal region in the gp120 V3 loop peptide treatment group, which may participate in the process of cognitive dysfunction. It has been proposed that one of the mechanisms whereby gp120 causes neurotoxicity was through a reduction in the neurotrophic factor environment crucial for cell survival. Another potential mechanism that may explain the neurotoxic effect of gp120 is its induction of TNFα and other inflammatory cytokines released from non-neuronal cells (Bazzi et al. 2001). TNFα, in turn, may cause apoptotic cell death, and consequently a decrease in BDNF. On the other hand, the up-regulation of the BDNF level found in HIV-1 patients was seen mainly in microglia. This may be a typical infection-induced inflammatory response evoked by neuronal injury, but not necessarily a cause. In addition, it has been reported that curcumin treatment, via up-regulation of BDNF, may reverse or protect hippocampal neurons from further damage (Xu et al. 2007). Here, we demonstrated that curcumin up-regulated the expression of BDNF in rats with cognitive dysfunction induced by gp120 V3 loop peptide. Our results combined with recent findings demonstrate that BDNF has a neuroprotective effect against gp120 V3 loop peptide mediated neuronal cell injury (Bachis et al. 2003). Further, the use of therapeutic agents such as curcumin, aimed at mimicking or increasing the normal activity of BDNF, may provide an effective strategy for this neurological disorder.

In summary, we provide evidence that HIV gp120 V3 loop peptide has a harmful impact on synaptic plasticity and cognitive function. Our findings suggest that curcumin supplementation may be an effective therapy to counteract the deleterious effects of gp120 V3 loop peptide on neuronal plasticity and function. Curcumin may provide an effective therapeutic strategy for HAD, and future experiments can further investigate the effects of curcumin on the pathological processes of this neurological disorder.

Conclusion

In this study we demonstrated that ICV administration of HIV-1 gp120 V3 loop peptide caused spatial learning and memory dysfunction, diminished LTP, and produced significant oxidative brain damage. The administration of curcumin, the principal curcuminoid of the Indian curry spice turmeric, significantly ameliorated HIV-1 gp120 V3 loop peptide-induced neuronal damage and/or dysfunctions, and up-regulated the expression of BDNF. These results indicate that curcumin supplementation may be an effective therapy to counteract the deleterious effects of gp120 on HIV-1-associated dementia.

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