HIV transactivator of transcription enhances methamphetamine-induced Parkinson's-like behavior in the rats

Zengxun Liu[a,b,*], Zhenchun Shi[b,*], Jintong Liu[a] and Yang Wang[a]

Abuse of methamphetamine (MA) increases the risk of infection of HIV-1, induces considerable neurotoxicity in several brain regions, and impairs the motor and cognitive function in individuals. HIV-1 transactivator of transcription (Tat) has also shown the potent capability to induce neuronal death and impaired brain function. The present study aims to study the synergistic effect of MA and Tat on cytokine synthesis in substantia nigra, striatal dopamine content, and behavioral performance in the rats. Although increased expression of cytokines (interleukin-1β and tumor necrosis factor-α) was observed in the substantia nigra in the rats receiving either MA or Tat alone, a combination of MA and Tat induced a larger and more sustained upregulation of cytokines. In the rats receiving either MA or Tat alone, significant loss in striatal dopamine content was found, which was further exacerbated in the rats receiving both MA and Tat. In the rats receiving either MA or Tat alone, significantly lower performance in the rotarod test and open-field test was observed, whereas the rats receiving both MA and Tat showed more sustained behavioral impairments. These results suggested that Tat protein synergized with MA to induce central neuroinflammation and impair the dopaminergic transmission, thus leading to sustained Parkinson's-like behavior. *NeuroReport* 25:860–864 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Exposure to methamphetamine (MA), a widely abused psychostimulant, induces considerable neurotoxicity in several brain regions and impairs motor and cognitive function in individuals [1]. A recent retrospective population-based large-scale cohort study reported a significantly increased risk for developing Parkinson's disease (PD) in individuals with a history of MA or amphetamine use [2]. Previous evidences have shown that exposure to MA induced significant oxidative stress resulting from the dysregulation of the dopaminergic system, hyperthermia, apoptosis, and neuroinflammation, thus mediating its neurotoxicity and impairments of brain function [1]. MA is a potent inducer of dopamine release and is toxic to dopamine neurons. Although significant neurodegeneration of dopaminergic terminals and neuroinflammation have been reported extensively in the striatum [3], the effect of MA on the cell bodies of dopaminergic neurons in substantia nigra remains disputed.

It is well known that abuse of MA is particularly high in groups that are at a higher risk for HIV-1 infection. Although HIV-1 itself does not infect neurons, viral proteins, such as transactivator of transcription (Tat) and glycoprotein 120, show potent neurotoxicity in the central neurons [4]. Exposure to Tat induced considerable synaptic degeneration, neuroinflammation, and neuronal death in the hippocampal CA1 area, striatum, and other brain regions [5]. Emerging evidences suggest that Tat may impair the dopamine system in the brain and potentiate the motor and cognitive dysfunction induced by MA in the rodent model [6]. The present study aims to investigate the synergistic effect of Tat and amphetamine on nigral neuroinflammation, striatal dopamine content, and motor function in a rodent model.

Materials and methods

Administration of methamphetamine

Adult male Wistar rats (weighing ~180–210 g) were obtained from the Institutional Center of Experiment Animals and were housed under standard laboratory conditions (22 ± 2°C and 12 : 12 h light cycle) with free access to food and water. All animal protocols were approved by the Institutional Animal Care and Use

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Committee and were carried out following the guidelines of National Institution of Health.

(+)-MA (Sigma-Aldrich, St. Louis, Mossouri, USA) hydrochloride was dissolved in 0.9% saline. (+)-MA (three doses of 10 mg/kg, at 3-h intervals) was injected intraperitoneally and saline in the same volume was injected into the rats in control group.

Cannula implantation and microinjection

The methods for site-specific cannula implantation and microinjection were similar to those reported previously [7]. After anesthetization with sodium pentobarbital solution (50 mg/kg), rats were placed in a stereotaxic frame and implanted with bilateral 26-G stainless-steel guide cannulas (Plastics One, Roanoke, Virginia, USA) into the substantia nigra (anteroposterior, −5.3; mediolateral, ±2.2; dorsoventral, −7.0) [7]. The guide cannula was then secured to the skull with dental cement and capped. After the implantation surgery, rats were allowed a recovery period of 1 week before subsequent experiments. The awake animals were administered an intranigral infusion of the recombinant HIV-1 protein Tat_{1-86} (10 μg/1 μl; Abcam, Cambridge, Massachusetts, USA) or scramble peptide (in same concentration) through a 33-G injector 30 min before the first injection of MA. All cannula placements for the substantia nigra were histologically verified afterward.

Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed to detect the concentration of cytokine interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in the substantia nigra. The substantia nigra tissues from the rats in all groups were collected and processed using commercial ELISA kits (R & D Systems, Minneapolis, Minnesota, USA) following the instructions provided by the manufacturer.

Measurement of dopamine contents in the striatum

The dopamine content in the striatum was measured following the protocols as described previously [8]. Briefly, after decapitation of animals, the brain was removed and tissues from the dorsal striatum were dissected out, frozen in liquid nitrogen, and stored at −80°C until assayed. Each frozen tissue sample was weighed and then homogenized in 200 μl of 0.2 M perchloric acid containing 100 ng/ml isoproterenol as an internal standard. The homogenate was placed on ice for 30 min and then centrifuged at 20 000*g* for 15 min at 4°C. Dopamine contents in the supernatants were measured with HPLC (Eicom, San Diego, California, USA) and expressed as μg/g tissue weight.

Motor performance testing

Motor performance was evaluated using a rotarod apparatus as described [9]. Before treatment with MA or Tat, the animals were placed in a rotarod with a 60-mm-diameter textured rod, 75 mm in length, rotating at a speed of 25 rpm for 5 days to ensure that they could maintain themselves on the rod for at least 180 s. Each animal was tested 5 times, with a 5-min interval between each trial, and the maximum duration of the test was 5 min. The time spent by the animal on the rotarod was considered as the latency to fall. All animals were tested at days 1, 3, 7, and 10 after the initial MA and/or Tat administration.

Locomotor activity

Locomotor experiments were conducted as described previously [10]. The locomotor chambers were 40 × 40 × 40 cm (Coulbourn Instruments, Whitehall, Pennsylvania, USA) and had clear plexiglas walls with a stainless-steel floor covered with a thin layer of pine-chip bedding. Photobeams were arranged in a 16 (*x*-axis) photocell array, spaced 2.54 cm apart. During each locomotor test, session (60 min), a 70 dB white noise was generated to mask any possible background noise. Locomotor activity was measured by recording the moving distance in centimeters. All animals were tested at days 1, 3, 7, and 10 after the initial MA and/or Tat administration.

Drugs and data analysis

MA, Tat, and all other reagents were purchased from Sigma, Abcam, or other commercial source. All data were presented as mean ± SEM and analyzed statistically using a *t*-test or analysis of variance, followed by post-hoc analysis. The criterion for statistical significance was $P < 0.05$.

Results

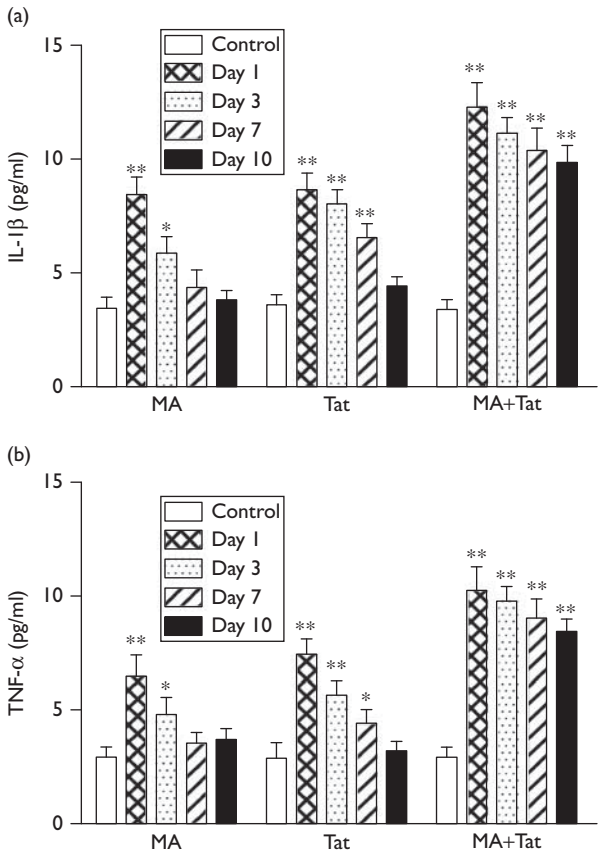
Tat enhanced the MA-induced upregulation of cytokines

As shown in Fig. 1, administration of MA significantly increased the nigral cytokines (IL-1β and TNF-α) at day 1 and day 3, which was recovered at day 7 and day 10. It was observed that administration of Tat (10 μg) into the substantia nigra also significantly upregulated the striatal cytokines (IL-1β and TNF-α) at days 1, 3, and 7, which was also recovered at day 10. Moreover, a combination of both treatments significantly further enhanced the upregulation of nigral cytokines at day 1 through day 10. These results suggested that coadministration of Tat significantly enhanced and extended MA-induced upregulation of cytokines synthesis in the substantia nigra.

Tat potentiated the MA-induced dopamine deficit

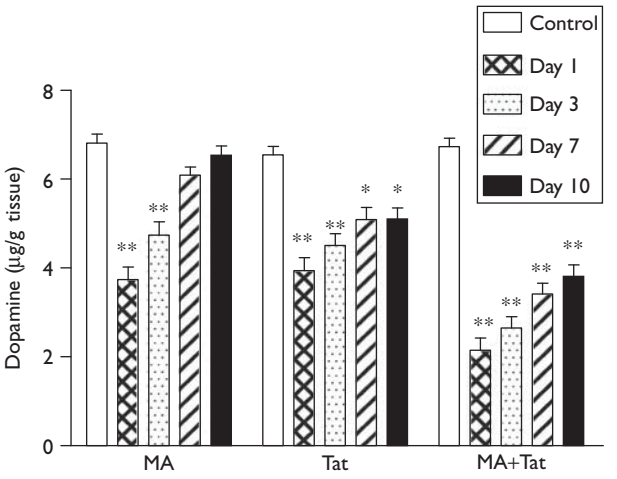
As shown in Fig. 2, administration of MA induced a significant reduction in dopamine content in the striatum at day 1 and day 3, which was eventually recovered at day 7 and day 10. It was also found that administration of Tat (10 μg) into the substantia nigra also significantly decreased the striatal dopamine at day 1 to day 10. Moreover, a combination of both treatments induced a further significant decrease in the striatal dopamine at day 1 through day 10. These results suggested that

Fig. 1



HIV Tat enhanced MA-induced upregulation of cytokines. (a) Administration of MA (3 × 10 mg/kg) or Tat (10 μg) alone significantly increased the IL-1β in the substantia nigra, whereas a combination of both treatments induced further upregulation and sustained expression of IL-1β ($N = 7–9$ rats per group); (b) administration of either MA or Tat significantly increased the TNF-α in the substantia nigra, whereas a combination of both treatments further increased the expression of TNF-α ($N = 7–9$ rats per group). $^*P < 0.05$; $^{**}P < 0.01$. MA, methamphetamine; Tat, transactivator of transcription.

Fig. 2



HIV Tat enhanced MA-induced striatal dopamine deficit. Administration of either MA or Tat reduced the dopamine content in the striatal, whereas a combination of both treatments induced further reduction in the dopamine content in the striatum ($N = 7–9$ rats per group). $^*P < 0.05$; $^{**}P < 0.01$. MA, methamphetamine; Tat, transactivator of transcription.

coadministration of Tat significantly exacerbated MA-induced reduction of striatal dopamine.

Tat potentiated the MA-induced behavioral impairments

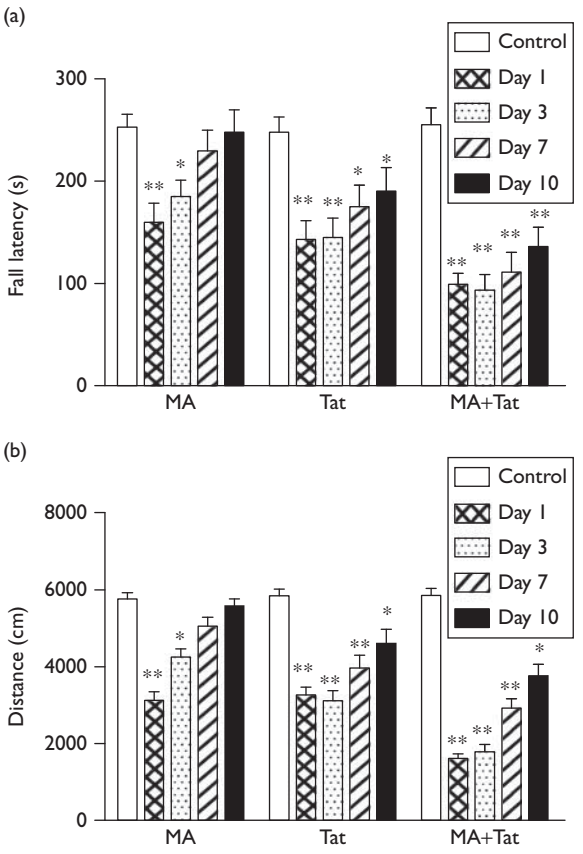
As shown in Fig. 3a, administration of MA significantly shortened the time that the rats spent on the rotarod at day 1 and day 3, which indicated an impaired motor balance. This impaired fall latency recovered gradually at day 7 and day 10. Meanwhile, delivery of Tat (10 μg) into the substantia nigra also significantly decreased the fall latency in the rats at day 1 to day 10. Notably, a combination of both treatments further decreased the fall latency at day 1 to day 10. These results suggested that coadministration of Tat significantly enhanced and extended MA-impaired motor balance.

As shown in Fig. 3b, rats treated with MA showed considerable reduction in moving distance in the open-field test at day 1 and day 3 after treatment, which was gradually recovered to the control level at day 7 and day 10. Similarly, a microinjection of Tat (10 μg) into the substantia nigra also significantly decreased the moving distance in the rats at day 1 through day 10. It was further found that coadministration of MA and Tat protein further reduced in the moving distance at day 1 through day 10. These results indicated that Tat may synergize with MA to impair the locomotor activity in the rodents.

Discussion

It was well recognized that individuals with a history of MA use had a higher risk of developing PD later in life [2], whereas the underlying mechanism remained disputed. A study with transcranial sonography found an abnormally bright and enlarged substantia nigra, a strong risk factor for developing PD, in individuals with a history of MA use [11]. Repeated administration of a high dose of MA led to a long-lasting reduction in dopamine uptake and dopamine content in the rat striatum [12]. Significant neuronal loss and depletion of striatal dopamine content were observed in the mice treated with MA [13]. A single high dose of MA induced considerable neuronal apoptotic death in the striatum [14] and substantia nigra [15], and a continuous injection of a low dose of MA induced long-term striatal dopamine depletion by destroying dopamine nerve fibers [16]. MA induced oxyradical stress, autophagy, and neurite degeneration in the midbrain neuronal cultures [17]. In the present study, we found that exposure to MA induced significant upregulation of cytokines (IL-1β and TNF-α) in the

Fig. 3



HIV Tat enhanced MA-impaired performance in the rotarod test and locomotor activity. (a) Administration of either MA or Tat decreased the fall latency, which was further decreased by the combination of both treatments. (b) Administration of either MA or Tat reduced the moving distance in the open-field test, which was further decreased by the combination of both treatments ($N = 9–11$ rats per group). $^{*}P < 0.05$; $^{**}P < 0.01$. MA, methamphetamine; Tat, transactivator of transcription.

substantia nigra, decreased the dopamine content in the striatum, and impaired the behavioral performance in the rotarod test and open-field test. These results confirmed the detrimental effect of MA on the function of dopamine neurons.

Accumulating evidences suggested that Tat largely mediated the neurotoxicity and functional impairments of the brain in patients infected with HIV-1. A single local administration of Tat led to a significant reduction in evoked dopamine release in the nucleus accumbens [18]. Induction of HIV-1 Tat in the transgenic mice reduced the number of apical dendritic spines, disrupted the distribution of synaptic proteins, and impaired synaptic plasticity in the hippocampal CA1 area, thus leading to cognitive dysfunction [5]. Tat protein decreased the expression and function of dopamine transporter in cell surface and dopamine uptake, by a PKC-dependent mechanism, in rat striatal synaptosomes [19]. Meanwhile, Tat also induced considerable microglia activation and synthesis of cytokines, impaired synaptic architecture, and neuronal death in the mice brain [20, 21]. In the present study, we found that administration of Tat significantly increased the synthesis of cytokines (IL-1β and TNF-α) in the substantia nigra, decreased the striatal dopamine content, and induced motor impairments in the rats, which indicated the detrimental effect of Tat on the function of dopamine neurons in the brain.

Previous studies have also implied that Tat protein may potentiate the neurotoxicity induced by illicit drugs. Tat and MA increased the release of matrix metalloproteinase-1 and urokinase plasminogen activator from cultured brain-derived cells [22]. It was reported previously that Tat and opiates synergized to reduce dendritic spine number and induce neuronal apoptotic death in several brain regions including the striatum [23]. It was also reported that Tat enhanced MA-induced reductions in striatal dopamine release and content [24] in a synergistic manner, and inhibition of TNF-α signaling largely attenuated the synergistic interaction between Tat and MA in the rodents [25]. In the present study, Tat protein significantly enhanced the magnitude, as well as extended the duration, of MA-induced nigral cytokines (IL-1β and TNF-α) synthesis and loss of striatal dopamine content, which indicates their synergistic effect in inducing neurotoxicity. Further behavioral studies also showed that Tat protein exacerbated MA-impaired performance in rotarod and open-field tests, indicating their synergistic effect in impairing the brain function and inducing Parkinson's-like behavior.

Although previous studies showed the distinguished, but somehow convergent, mechanisms underlying MA-induced or Tat alone-induced neurotoxicity in the rodent model, there were evidences to show that a combination of both reagents synergistically exacerbated the oxidative stress, microglia activation, and cytokine synthesis in the cortical, hippocampal, and striatal regions of the brain in rodent models [1]. This might contribute toward the synergistic effect between MA and Tat in inducing more significant and sustained cellular and behavioral impairments in the rat in the present study.

Acknowledgements

Z.L. and Z.S. carried out the studies and contributed toward the manuscript. Z.L., J.L., and Y.W. designed the study and analyzed the data. J.L. and Y.W. supervised the study and finalized the manuscript. This work was supported by a grant from the Natural Science Foundation of Shandong Province (ZR2012HM026).

Conflicts of interest

There are no conflicts of interest.

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