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The overexpression of Thioredoxin-1 suppressing inflammation induced by methamphetamine in spleen

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ABSTRACT

Background: Methamphetamine (METH) is an addictive psychostimulant and has been shown to induce oxidative stress and inflammation in various tissues. Thioredoxin-1 (Trx-1) plays the roles in regulating redox and inhibiting inflammation. Whether Trx-1 is involved in METH-induced inflammation is still unknown.
Methods: The present study was designed to investigate inflammatory factors in spleen of wild type and Trx-1 overexpression transgenic mice after METH treatment.
Results: We found the mRNA level of Trx-1 was decreased and mRNA level of Trx-1 binding protein-2 (TBP-2) was increased. The mRNA levels of tumor necrosis factor-α (TNF-α), interferon-γ(IFN-γ), interleukin-2 (IL-2), T-bet and signal transducer and activators of transcription 4 (STAT 4) were increased and the mRNA levels of IL-10, GA-TA-binding protein-3 (GATA-3) and STAT 6 were decreased. Overexpression of Trx-1 reversed the above effects induced by METH.
Conclusion: The present study showed for the first time that Trx-1 overexpression suppressed the inflammation induced by METH.

1. Introduction

Methamphetamine (METH) is a highly addictive psychostimulant and has pleotropic effects on the immune response in diverse tissues (Peerzada et al., 2013). Thus, METH adversely impacts immunological responses, which might contribute to the higher rate and more rapid progression of certain infections, such as human immunodeficiency virus (HIV), hepatitis A, B, and C in drug abusers (Loftis et al., 2011). T cells play an important role in the body's immune response, particularly helper T cells (Th). Th cells are differentiated into Th1 and Th2 cells and secrete Th1/Th2 cytokines upon various antigen stimulations, such as interleukin-2 (IL-2), interferon-γ (IFN-γ), interleukin-4 (IL-4), tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10). Th1/Th2 cell differentiation is regulated by transcriptional factors, such as T-box expressed in T cells (T-bet), signal transducer and activator of transcription 4 (STAT4) and STAT6, GA-TA-binding protein-3 (GATA-3).

Thioredoxin-1 (Trx-1) is a redox regulating protein and highly conserved in mammalian systems. It plays the roles in growth promotion, neuroprotection, inflammatory modulation and inhibition of apoptosis (Loftis et al., 2011). The activity of Trx-1 regulating immune function is usually associated with its redox state (Griffiths et al., 2014). However, whether Trx-1 is involved in regulating the inflammatory responses induced by METH has not been reported. The aim of present study is to examine the inflammatory mediators and transcriptional factors in spleen of the wild type mice and Trx-1 overexpression transgenic mice after METH treatment. Our results showed Trx-1 overexpression reversed effects induced by METH. Thus, Trx-1 may play an important role in regulating immune dysfunction by METH.

2. Materials and methods

2.1. Reagents

METH was obtained from Yunnan Province Public Security Department and dissolved in saline. Anti-mouse Trx-1 polyclonal antibody was obtained from (Redox

Abbreviations: ASK1, Apoptosis signal-regulating kinase 1; CNS, Central nervous system; DC, Dendritic cell; GATA3, GA-TA-binding protein-3; HIV, Human immunodeficiency virus; HL-60, Human promyelocytic leukemia cell line; IFN-γ, Interferon-γ; IL-1β, Interleukin-1β; IL-2, Interleukin-2; IL-4, Interleukin-4; IL-10, Interleukin-10; LPS, Lipopolysaccharide; METH, Methamphetamine; NK, Natural killer; PAG, Proliferation-associated gene; qRT-PCR, Quantitative real-time polymerase chain reaction; STAT4, Signal transducer and activators of transcription 4; STAT6, Signal transducer and activators of transcription 6; T-bet, T-box expressed in T cells; TBP-2, Thioredoxin-binding protein-2; Tg, Transgenic; Th, Helper T cell; TNF-α, Tumor necrosis factor-α; Trx-1, Thioredoxin-1; VDUP1, Vitamin D3-up-regulated protein 1; WT, Wild type.

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Bioscience, Inc. Kyoto, Japan). This antibody does not cross react with human protein. Anti-mouse β-actin monoclonal antibody was supplied by (Santa Cruz Biotechnology, Inc. CA, USA).

2.2. Animals

Male C57BL/6 wild-type (WT) and C57BL/6 human Trx-1 transgenic (Trx-1 Tg) mice, 7–8 weeks of age, were used in the experiments. The mice were housed in plastic cages and kept on a 12 h light-dark cycle and had free access to food and water. The animals were cared for and used in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. C57BL/6 human Trx-1 transgenic (Trx-1 Tg) mice were constructed by (Cyagen Biosciences Inc. Guangzhou, China). All animal experiments were approved by the local Committee on Animal Use and Protection (No. LA2008305).

2.3. Methamphetamine treatment

Mice were divided into four groups ($n = 9$ per group). Wild type and Trx-1 Tg mice of non-METH groups were administered saline, once daily for 7 days. Based the studies on METH induced conditioned place preference (CPP), the dose of the METH is 0.25–2.5 mg/kg or above (Berry et al., 2012; Qi et al., 2009; Shen et al., 2010). We selected the concentration of METH 2.5 mg/kg in our study. WT and Trx-1 Tg mice of METH groups were received four intraperitoneal injections of 2.5 mg/kg METH on days 1, 3, 5, 7, three intraperitoneal injections of saline on days 2, 4, 6. After METH induced CPP, mice were sacrificed 2 h after the last treatment by cervical vertebra dislocation, and then heart perfusion was performed by using saline. The spleen was rapidly dissected out, frozen, and stored in a deep freezer at −80 °C until the assays.

2.4. Real-time polymerase chain reaction

Total RNA was extracted from 0.1 g spleen tissue by using a Trizol reagent kit (CWBIO Corporation, Beijing, China) and converted to cDNA by using the Revert Aid TM First Strand cDNA Synthesis Kit (Fermentas Walldorf Baden, Germany). The product was analyzed by using a Prism 7300 Sequence Detection System (Applied Biosystems, Foster, CA, USA). The following primer pairs were selected for real-time polymerase chain reaction (qRT-PCR): mouse β-actin F:5′-CAG TTC GCC ATG GAT GAC GAT-3′, R 5′-ATC TGG GTC ATC TTT TCA CGG TTG-3′; mouse IL-2 F: 5′-AGC TCT ACA GCG GAA GCA CA-3′, R:5′-CTC CTC AGA AAG TCC ACC AC-3′; mouse IFN-γ F:5′-AGA CTG TGA TTG CGG GGT TG-3′, R 5′-ACA TTC GAG TGC TGT CTG GC-3′; mouse TNF-α F:5′-GCC TAT GTC TCA GCC TCT TCT C-3′, R 5′-TGG GAA CTT CTC ATC CCT TTG G-3′; mouse L-10 F: 5′-GGT TGC CAA GCC TTA TCG G-3′, R: 5′-ACC TGC TCC ACT GCC TTG CT-3′; mouse T-bet F: 5′-CCT GTT GTG GTC CAA GTT-3′, R: 5′-TTT CCA CAC TGC ACC CAC TT-3′; mouse STAT4 F: 5′-ATC CCC TAG AGG AAT GGC GT-3′, R: 5′-GAG CTA GCA GAT GCC GGA TT-3′; mouse GATA3 F: 5′-CCT ATG TGC CCG AGT ACA GC-3′, R:5′-GCA GGC ATT GCA AAG GTA GT T-3′; mouse STAT6 F: 5′-CGC TGA TAA GCC GTC TGG AT-3′, R: 5′-GCT CTG ACC ATT GGA GGC TT-3′; mouse Trx-1 F: 5′-ATG GTG AAG CTG ATC GAG AGC-3′, R: 5′-GGC ATA TTC AGT AAT AGA GGC-3′; mouse TBP-2 F: 5′-GCT GGA CGA TGT GGA CGA C-3′, R: 5′-AGA AAT GCG CTA ATA CAG ATG CT-3′. Reaction mixtures containing Premix Ex Taq TM (TaKaRa code: DRR039) and SYBR Green (TaKaRa code: DRR041; TaKaRa Biotechnology, Dalian, China) were prepared according to the manufacturer's protocol. β-actin was used as an internal standard for all samples.

2.5. ELISA analysis

The spleen tissue was weighed (30 mg) and homogenized in stroke-physiological saline solution (270 μl). Samples were spun at 3000 rpm for 15 min at 4 °C. The supernatant was aliquoted and stored at −80 °C for future study.

Malonaldehyde (MDA) was quantified by using mouse MDA ELISA kit (Multiscience CO. LTD, Hangzhou, China) according to the manufacturer's instructions. Read the Optical Density (O.D.) at 450 nm on a Bio-Rad Benchmark plus microplate spectrophotometer (Hercules, CA, USA) within 15 min. The values thus obtained were plotted into the standard plot prepared by using serial dilutions of the standard provided with the kit and MDA concentration was calculated. MDA was expressed in nmol/ml of protein.

The concentrations of IL-2, IL-10 and IFN-γ were quantified by using mouse ELISA Cytokine Kit (BD OptEIA™; BD Biosciences, CA, USA) and determined the optical density by using a Bio-Rad Benchmark plus microplate spectrophotometer (Hercules, CA, USA) to 450 nm. The values thus obtained were plotted into the standard plot prepared by using serial dilutions of the standard provided with the kit and concentrations of cytokines were calculated. The concentrations of IL-2, IL-10 and IFN-γ were expressed in pg/ml of protein.

2.6. Western blot

Protein lysates were prepared by using a solubilizing solution (20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% NP-40, 1 mM EDTA, 1 mM phenylmethanesulfonyl fluoride, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM Na3VO4, 1 mM β-glycerol phosphate, and 1 mg/ml leupeptin). Protein concentration was determined by using the Bio-Rad protein assay reagent (Hercules, CA, USA). Equal quantities of proteins were separated by 15% (for Trx-1 and β-actin) SDS-PAGE and transferred to the polyvinylidene difluoride membrane (Millipore Corp., Billerica, MA, USA). The membrane was soaked in 10% skim milk (in phosphate buffered saline, pH 7.2, containing 0.1% Tween 20) or 3% bovine albumin V (in Tris-buffered saline, pH 7.2, containing 0.1% Tween 20) overnight at 4 °C and then incubated with primary antibodies (1:1000) followed by peroxidase-conjugated anti-mouse or anti-rabbit IgG (1:10,000) (KPL, Gaithersburg, MD, USA). The epitope was visualized by an ECL Western blot detection kit (Millipore). Densitometry analysis was performed by using Image J software.

2.7. Statistical analysis

Data were expressed as means ± SD values. Statistical analysis was performed by using SPSS software. The one-way ANOVA followed by a post hoc multiple comparison test was used to compare wild type and METH treated groups. A P value of <0.05 was considered as statistically significant.

3. Results

3.1. Overexpression of Trx-1 suppressed the expressions of IL-2, IFN-γ and TNF-α induced by METH treatment in spleen

It has been known that METH disrupts immune homeostasis and induces the proinflammatory response (Harms et al., 2012, Permpoonputtana and Govitrapong, 2013). The spleen is an important organ of immunoregulation. We first detected mRNA levels of IL-2, IFN-γ and TNF-α in spleen of wild type and Trx-1 Tg mice by using qRT-PCR. As we expected, the mRNA levels of IL-2, IFN-γ and TNF-α were increased in spleen after METH treatment and these increases were suppressed in Trx-1 Tg mice (Fig. 1A, B and C). The protein levels of IL-2 and IFN-γ were also increased by METH and these increases were repressed in Trx-1 Tg mice (Fig. 1D and E).

3.2. Overexpression of Trx-1 reversed the decrease of IL-10 by METH treatment in spleen

When the proinflammatory mediators are increased, Th2-specific factors are decreased significantly. IL-10 is one of the Th2-specific factors. We detected the level of IL-10 in spleen after METH treatment. We found that the mRNA or protein level of IL-10 was decreased and the decrease was reversed in Trx-1 Tg mice (Fig. 2A and B).

3.3. Overexpression of Trx-1 suppressed the expressions of T-bet and STAT 4 after METH treatment in spleen

To further investigate whether expressions of IL-2, IFN-γ and TNF-α are induced by METH via the transcriptional factors, the mRNA levels of T-bet and STAT 4 were detected. The mRNA levels of T-bet and STAT4 were increased by METH when compared to WT wild type group, however the mRNA levels of T-bet and STAT4 in Trx-1 Tg mice after METH treatment were not increased when compared to Trx-1 Tg mice (Fig. 3A and B). These data showed that the expressions of IL-2, IFN-γ and TNF-α were associated with transcriptional factors after METH treatment. The overexpression of Trx-1 inhibited the increases of T-bet and STAT4 induced by METH.

3.4. Overexpression of Trx-1 reversed the expressions of GATA-3 and STAT6 after METH treatment in spleen

We further detected the expressions of GATA-3 and STAT 6, which regulate IL-10 expression. The mRNA levels of GATA-3 and STAT 6 were decreased and the decreases were reversed in Trx-1 Tg mice (Fig. 4A and B). These data showed that the expression of IL-10 was associated with transcriptional factors after METH treatment and overexpression of Trx-1 reversed these effects.