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Original research article

The influence of AAV2-mediated gene transfer of human IL-10 on neurodegeneration and immune response in a murine model of Parkinson's disease

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ABSTRACT

Background: The aim of this study was to examine the effect of AAV2-hIL-10 (vector containing cDNA for human interleukin 10) on dopaminergic system activity (measured as DA levels and TH mRNA expression in mouse striata), and other monoamine and amino acid neurotransmitters concentration as well as development of inflammatory processes (measured as TGF-β, IFN-γ and GFAP mRNA expression) in a murine MPTP neurotoxicant model of Parkinson's disease.
Methods: Male C57BL/6 mice 12 months-old were used in this study. AAV2-hIL-10 vector was bilaterally administered into striatum at 14, 21 or 28 days prior to MPTP intoxication. Animals were sacrificed at 7 days following MPTP injection. The expression of hIL-10 (human interleukin 10) was examined by ELISA. Striatal monoamine and amino acid neurotransmitters were measured by HPLC method. TH, TGF-β, IFN-γ and GFAP mRNA expression was examined by RT-PCR method.
Results: MPTP treatment dramatically reduced DA levels and decreased TH mRNA expression in mouse striata, effects that were significantly impeded by AAV2-hIL-10 administration prior to MPTP intoxication. AAV2-hIL-10 infusion increased IFN-γ, TGF-β and GFAP mRNA expression.
Conclusions: Our data suggest that the transfer of AAV2-hIL-10 into the striatum may play a neuroprotective role in the mouse MPTP model of PD and these effects are mediated by the anti-inflammatory action of IL-10.
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Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder of unknown etiology, characterized in part by a selective, abnormal loss of nigrostriatal dopaminergic neurons of the substantia nigra pars compacta (SNpc), and loss of dopamine (DA) in the striatum [1,2]. Degeneration of the nigrostriatal pathway is also accompanied by inflammation observable as an increased synthesis of pro-inflammatory cytokines such as IL-1β, IL-2, IL-4, IL-6, TNFα, IFN-γ. This inflammation is thought to be an important factor in loss of dopaminergic neurons [3]. Neurodegenerative mechanisms activated by inflammatory factors may contribute to mitochondrial dysfunction, oxidative stress, leading

Abbreviations: 5HIAA, 5-hydroxyindoleacetic acid; 5HT, 5-hydroxytryptamine; AAV, adeno-associated virus; AAV2-GFP, AAV2-green fluorescent protein; AAV2-hIL-10, vector containing cDNA for human interleukin 10; ASP, aspartic acid; cDNA, complementary deoxyribonucleic acid; DEPC, diethyl pyrocarbonate-treated water; DA, dopamine; DOPAC, dihydroxyphenyl acetic acid; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GFAP, glial fibrillary acidic protein; GLU, glutamic acid; hIL-10, human interleukin 10; HVA, homovanillic acid; HPLC, High Performance Liquid Chromatography; IFN-γ, interferon gamma; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MMLV, moloney murine leukemia virus; mRNA, messenger ribonucleic acid; PD, Parkinson's disease; qRT-PCR, quantitative reverse transcription polymerase chain reaction; SNpc, substantia nigra pars compacta; ST, striatum; TGF-β, transforming growth factor beta; TH, tyrosine hydroxylase.

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to apoptosis of dopaminergic neurons [4]. An increased expression of inflammatory factors is accompanied by a reduction of neurotrophins, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), as well as increased expression of TGF-α and TGF-β [5].

A potential therapeutic strategy for PD is to limit development of the inflammatory response [6–8] through local production of the anti-inflammatory cytokine interleukin-10 (IL-10) that modulates the biological activity of immune cells, thus decreasing production of pro-inflammatory mediators including cytokines, chemokines and adhesion molecules. IL-10 is produced by TH2 cells, macrophages, keratinocytes, normal B cells and LY-1+ (CD5+) cells [9,10], and it potently antagonizes the actions of major inflammatory cytokines. Biologically active IL-10 protein is a homodimeric, pleiotropic cytokine that is expressed in the CNS by monocytes, astrocytes and microglia [11]. IL-10 has a short half-life and is not able to cross the blood-brain barrier [12]; therefore the viral vectors may be the best way to achieve sustained high expression of inflammatory cytokines within the striatum. We have previously shown that adeno-associated virus 2 (AAV2) encoding human IL-10 is protective in the rat 6-OHDA model [13]. This study extends these observations to the mouse MPTP model of Parkinson's disease. Mice afford many more opportunities to study neuro-inflammation than do rats because of the wide range of genetically modified animals available that thereby permit mechanistic investigations of this phenomenon. We show here that hIL-10 protects mice significantly against MPTP toxicity and that this effect is evident not only in terms of dopamine but also with respect to other monoamine and amino acid neurotransmitters as well as some markers of inflammatory response. Our study establishes parameters for further investigations of neuroinflammation and PD in a flexible mouse model.

Materials and methods

Animals

Male C57BL/6 mice, 12 months-old, 35–40 g, were used in this study. Animals were housed in standard laboratory condition under 12-h light/dark cycle (7:00 am/7:00 pm), in controlled temperature (22 ± 5 °C) and 60 ± 5% humidity. All animals were given free access to food and water. The experimental protocols were approved by the 2nd Local Ethic Committee in Warsaw and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and in accordance with *EU Directive 2010/63/EU for animal experiments.* All efforts were made to reduce the number of animals used and to minimize animals suffering.

Experimental protocol

AAV2-hIL-10 vector administration – stereotactic surgery

AAV2-hIL-10 (vector containing the complementary DNA for the human interleukin 10) and control vector AAV2-GFP (AAV2-green fluorescent protein; GFP) as a reporter transgene were used in this study as previously described [13].

Under surgical anesthesia (ketamine: ksylazyne; 1:1; 2 ml/kg), mice were placed in a stereotactic frame (Stoelting). An incision was made in the skin overlying the skull and a burr-hole was made with a needle just above the infusion site. A programmable microsyringe pump (Micro 4; World Precision Instruments) was used to deliver 2 μl of vector into the striatum at the following stereotactic coordinates: AP: +0.62, ML: ±1.75 relative to bregma and DV: −3.5 mm relative to dura [14]. AAV2-hIL-10 vector [2×10^{10} vector genomes (vg)] was bilaterally administered at 0.5 μl/min into striatum. After infusion, the scalp was closed with sutures.

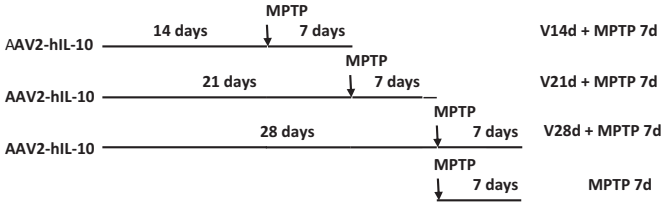

Fig. 1. A scheme of the experiment. V14d + MPTP7d – AAV2-hIL-10 injected group 14 days previous MPTP intoxication; V21d + MPTP7d – AAV2-hIL-10 injected group 21 days previous MPTP intoxication; V28d + MPTP7d – AAV2-hIL-10 injected group 28 days previous MPTP intoxication; MPTP7d – MPTP injected group.

MPTP intoxication

MPTP hydrochloride (MPTP-HCl; Sigma–Aldrich) was dissolved in 0.9% sterile saline and was injected into the animals in four intra-peritoneal (i.p.) injections at 1-h intervals using a dose of 10 mg MPTP-HCl/kg body weight, to the total dose of 40 mg/kg. Control animals were injected with an equivalent volume of 0.9% NaCl. MPTP was injected 14, 21 or 28 days post AAV2-hIL-10 vector administration (Fig. 1). Six to eight mice were killed at each experimental group (MPTP7d – MPTP injected group; V14d + MPTP7d – AAV2-hIL-10 injected group 14 days previous MPTP intoxication; V21d + MPTP7d – AAV2-hIL-10 injected group 21 days previous MPTP intoxication; V28d + MPTP7d – AAV2-hIL-10 injected group 28 days previous MPTP intoxication). Animals were euthanized by spinal cords dislocation at the 7 day following MPTP intoxication.

ELISA

Initially, the expression of hIL-10 in striatum was determined with a commercially available human Interleukin-10 ELISA Kit (AssayMax® ASSAY PRO). Human IL-10 was measured in the striatal extracts of control mice and at 7 (V7d), 14 (V14d), 28 (V28d) and 35 (V35d) days after AAV2-hIL-10, and 35 days after a control AAV2-GFP (P35d) infusion. Each experimental group consisted of 4–6 animals. Mice were euthanized and striata micro-dissected, weighed, frozen rapidly and stored at −80 °C until further use. Tissue samples were later homogenized (Virsonic 60; Virtis Inc.) and centrifuged at 13,000 × *g* for 15 min at 4 °C. The supernatant solution was used for assay of hIL-10. Plates were read on a ELx800 Universal Microplate Reader (Bio-Tek, USA) and results expressed as pg hIL-10/mg wet weight of tissue.

Immunohistochemistry

35 days after AAV2-hIL-10 infusion the striatal distribution of hIL-10 was assayed by immunohistochemistry. Animals (6 per group) were anaesthetized with sodium pentobarbital (50 mg/kg) and transcardially perfused with heparinized 0.9% NaCl solution followed by 10% paraformaldehyde-lysine periodate fixative (PLP). The brains were removed, post-fixed in PLP for 4–6 h, then immersed in a 20% sucrose solution overnight at 4 °C and rapidly frozen. Horizontal sections (20 μm) of the ST and the substantia nigra (SN) were cut on a cryostat, transferred onto gelatinized slides, and stored at −20 °C until further processed. Sections were incubated for 48 h with an antibody against a hIL-10 (goat anti-hIL-10, R&D System) diluted 1:20 in 0.1 M PBS with 0.1% bovine serum albumin (BSA) and 0.1% Triton X 100 followed by a 2 h incubation with biotinylated secondary rabbit anti-goat IgG (Jackson ImmunoResearch). Binding of the primary antibody was revealed by the avidin-biotin-peroxidase-DAB method (Vectastain kit, Vector Laboratories, USA). The slides were dehydrated, cleared in xylene and mounted. Sections were examined with an Olympus BX53 microscope in conjunction with Olympus CAST software (Olympus, Albertslund, Denmark).

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