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ScienceDirect
Neuroscience Letters 418 (2007) 175–180
Neuroscience Letters
www.elsevier.com/locate/neulet

Mutant SOD1^{G93A} in bone marrow-derived cells exacerbates 3-nitropropionic acid induced striatal damage in mice

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Received 8 January 2007; received in revised form 28 February 2007; accepted 10 March 2007

Abstract

3-Nitropropionic acid (3-NP), an irreversible inhibitor of succinate dehydrogenase, produces selective lesions in striatal neurons that resemble those observed in Huntington's disease neuropathology. In this study, we evaluated the role of peripheral bone marrow-derived cells (BMDCs) in the 3-NP-induced striatal damage by transplanting bone marrow cells with human SOD1 G93A mutation (mSOD1^{G93A}) which induces amyotrophic lateral sclerosis through an unknown gain of toxicity and mitochondrial dysfunction. We assessed striatal damage after 3-NP treatment in the recipient C57BL/6 wild-type (WT) mice that received bone marrow cells from WT or mSOD1^{G93A} transgenic donor mice (WT → WT or mSOD^{G93A} → WT). After intraperitoneal injection of 3-NP, six of the eight mSOD1^{G93A} → WT mice had bilateral striatal lesions while only one out of eight WT → WT mice had a striatal lesion. The lesion volume was significantly higher in the mSOD1^{G93A} → WT mice than in the WT → WT mice. However, following an intrastriatal injection of 3-NP, there was no significant difference in the lesion volumes between the WT → WT mice and mSOD1^{G93A} → WT mice. Thus, the exacerbation of 3-NP-induced striatal damage in mSOD^{G93A} → WT mice was only seen after systemic administration of 3-NP, but not after intrastriatal injection. These results demonstrate that altered SOD1 activity (mSOD^{G93A}) in BMDCs affects striatal damage probably through a mechanism involving a systemic factor.

Keywords: Bone marrow-derived cells; 3-Nitropropionic acid; G93A SOD1 transgenic mice; Striatum; Huntington's disease

3-Nitropropionic acid (3-NP) is a natural environmental toxin made by various plants and fungi. Accidental ingestion of contaminated food in humans and cattle produces acute encephalopathy which selectively lesions the basal ganglia [18,21]. Systemic injection of 3-NP to non-human primates and rodents produces selective degeneration of striatal medium spiny neurons and mimics some features of the neuropathology observed in Huntington's disease (HD) and is thus widely used as a neurotoxin model of HD [6]. The mechanisms underlying the selective striatal damage by 3-NP remain unknown. The primary biochemical basis for 3-NP toxicity is irreversible inhibition of the tricarboxylic acid cycle and respiratory chain enzyme succinate dehydrogenase (SDH). Theoretically, irreversible inhibition of SDH following systemic administration of the toxin would lead to an ATP deficiency in all tissues and cells containing SDH in the body. However, while the inhibition of SDH activity for a given dose of 3-NP is homogeneously distributed throughout the brain, the neurodegenerative effects are preferentially localized within the striatum [5]. Several hypotheses, including glutamate excitotoxicity [3,17], dopamine [25] and anatomic vulnerability of the lateral striatal artery, as well as disruption of the blood brain barrier [26,16,17], have been proposed as potential mechanisms for the selective striatal lesions induced by 3-NP treatment.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by the progressive loss of motor neurons in the spinal cord and brain. Transgenic mice with over-expression of human SOD1 carrying the G93A mutation (mSOD1^{G93A}) develop a rapidly progressive muscular weakness due to motor neuron degeneration and are used as animal models of ALS [15]. The mechanisms by which mSOD1^{G93A} causes disease have been mainly attributed to an unknown gain of toxicity of the mutant enzyme and mitochondrial dysfunction [7]. Interestingly, recent studies suggest that wild-type (WT) bone marrow-derived cells (BMDCs) [10] and microglial cells [8] can ameliorate the phenotype of mSOD1^{G93A} mice. These

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doi:10.1016/j.neulet.2007.03.038

findings are indicative of the involvement of mSOD1^{G93A} toxicity in BMDCs in the development of motor neuron degeneration.

Consistent with the notion of the involvement of BMDCs in motor neuron degeneration, our recent study [20] indicates that adenosine A_{2A} receptor activity in BMDCs plays an important role in 3-NP induced striatal damage. We have demonstrated that altered activity of the A_{2A} receptor in BMDCs (by transplanting bone marrow cells from global A_{2A}R KO mice into wild-type C57BL/6 mice) modifies the functional and neurochemical outcomes of 3-NP treatment. Because mSOD1^{G93A} mice have increased vulnerability to 3-NP [2], and mutant SOD1 is ubiquitously expressed in mSOD1^{G93A} mice including BMDCs [4], we hypothesized that altered BMDC activity (transplanting bone marrow cells containing "gain-toxicity" and dysfunctional mitochondria from mSOD1^{G93A} mice) contributes to the 3-NP toxicity. In this study, we examined the effects of selective overexpression of mSOD1^{G93A} in BMDCs on the extent of 3-NP neurotoxicity after systemic or intrastriatal injection of 3-NP.

Hemizygote male G93A mice [B6SJL-TgN(SOD1-G93A)1Gur; the Jackson Laboratory, Bar Harbor, ME] were bred with littermate controls to produce transgenic mice. The genotype of the transgenic mice was determined by PCR assay of tail DNA with the primers recognizing human SOD1 [2]. Transgenic mSOD1^{G93A} mice had the typical pathological process with death at average age of 145 days.

Chimeric mice were produced using bone marrow transplantation (BMT) as described previously [20,30]. Briefly, male C57BL/6 mice from Charles River (age, 6–9 weeks) were lethally irradiated with a total dose of 12.5 Gy from a ^{137}Cs source. After irradiation, the mice received transplantation of bone marrow cells (2–3 million per 0.2–0.3 ml) via tail vein injection. Bone marrow cells were isolated from female mSOD1^{G93A} mice (age, 105–115 days) and WT mice (age, 90–120 days) by flushing the tibia and femur with RPMI 1640 medium under sterile conditions. The reconstitution efficacy was assessed by three independent methods which have been described in details previously [20,30]. First, during each irradiation, two control mice did not receive BMT and died within 10 days. Second, we took advantage of sex mismatched BMT in the study (from female donor to male recipient); 7 weeks after BMT, genotyping of sex chromosome-linked genes (*Jarid1d* and *Jarid1c*) was performed on peripheral blood mononuclear cells (PBMCs). A single primer pair amplifies both the X-linked and the Y-linked genes but yields different sized products as described previously [20,30]. Third, we used a CD45.1 variant substrain of C57BL/6 mice (B6.SJL-*Ptprca Pep3b*/BoyJ) as donor source. The CD45.1 epitope is absent in the recipient mice. Seven weeks after the transplantation, PBMCs were isolated from the chimeric mice and CD45.1-positive cells were detected with PE-conjugated anti-mouse CD45.1 monoclonal antibody (BD Pharmingen) and flow cytometry (BD Pharmingen). The percentage of CD45.1-positive cells was $89.1 \pm 3.1\%$ in PBMCs of chimeric mice 7 weeks after transplantation [20].

Seven months after the transplantation, mice were subjected to 3-NP injection and were monitored for neurological deficit signs as described previously [20]. 3-NP (5 mg/ml, Sigma) was dissolved in saline and the pH was adjusted to 7.4 with 1N NaOH. 3-NP was injected intraperitoneally twice daily for 2 days at 12 h intervals (8:00 a.m. and 8:00 p.m.) at a dose of 60 mg/kg on the first day and 70 mg/kg on the second day (i.e., a 60–60–70–70 dose regime). For intrastriatal injection, 3-NP was dissolved in PBS, adjusted to pH 7.4, and filtered (Millipore 0.22 μm). A 0.5 μl of PBS containing 50 nmol 3-NP was injected at a 5 μl/h rate into the left striatum at the coordinates of 0.9 mm anterior, 2 mm lateral, and 2.5 mm deep from bregma, and the needle was left in place for an additional 3 min before being slowly withdrawn. An equal volume of PBS was administrated to the right striatum as a control.

Twenty-four hours after the last 3-NP intraperitoneal injection or twenty-four hours after intrastriatal injection, mice were anesthetized with Tribromoethanol (Avertin, 125 mg/kg) and perfused transcardially with saline followed by ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were immediately removed and post-fixed overnight in the same fixative, then cryoprotected in 30% sucrose in 0.1 M phosphate buffer (pH 7.4). Sequential coronal sections (30 μm) were made on a freezing microtome, starting from the anterior aspect of the corpus callosum (bregma = 1.40 mm) throughout of the entire striatum (bregma = −1.30 mm), according to the mouse brain atlas [19]. For histological assessment, every sixth section (∼210 μm interval) was processed for cresyl violet staining or Fluoro-Jade C staining to assess cell loss and neuronal degeneration. Cresyl violet staining was performed with standard protocols. The method for Fluoro-Jade C staining (Fluoro-Jade C, Histo-Chem, Jefferson, AR) has been described previously [20,27]. Analysis of the lesion volumes was performed by digitally acquiring images (Spot Insight, Diagnostic Instruments) of cresyl violet stained sections through the striatum with 4× objective. The lesion area was characterized by extensive neuronal loss as determined on cresyl violet-stained sections. Lesion volumes were calculated by summing the cross-sectional areas of the lesion in each section and multiplying this value by the distance between sections. Fluoro-Jade C staining was only assessed in sections from the core of the striatal lesion. For IgG immunohistochemical staining, we used biotinylated goat anti-mouse IgG (1:200, Jackson ImmunoResearch Laboratories) as primary antibody. After reaction with avidin and biotinylated horseradish peroxidase macromolecular complex (Vectastain ABC Elite kit, Vector Laboratories), the peroxidase activity was visualized using DAB as a chromogenic substrate (Vector Laboratories).

Following the bone marrow transplantation, the chimeric mice (WT → WT and mSOD^{G93A} → WT) were observed for 7 months. Mice behaved normally and did not display limb weakness or any other signs of motor neuron disease. Histological examination of the spinal cord at 7 months after BMT demonstrated that there was no difference between the two kinds of chimera. Thus, while wild type BMDCs can ameliorate the ALS phenotype [10], mSOD^{G93A} in BMDCs was not sufficient to induce any ALS motor symptoms, similar results as reported by Beers et al. [4].

At 7 months post-transplantation, WT → WT mice (26.1 ± 0.4 g) and mSOD1^{G93A} → WT mice (26.3 ± 0.5 g) received four doses of 3-NP. After the first two injec-