Neuroprotective effects of diallyl trisulfide in SOD1-G93A transgenic mouse model of amyotrophic lateral sclerosis

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ABSTRACT

Diallyl trisulfide (DATS) is one of the major constituents in garlic oil and has been documented to transcriptionally activate phase II enzymes. The purpose of this study is to evaluate the effects of DATS in prolonging disease duration and survival in a transgenic mouse model of amyotrophic lateral sclerosis (ALS). SOD1-G93A transgenic mice were randomly divided into DATS-treated group (80 mg/kg/d, p.o.) and vehicle-treated group at disease onset stage. Oral administration of DATS beginning at clinical onset stage significantly prolonged disease duration and extended life span for about one week. DATS treatment induced HO-1 and reduced GFAP expression in the lumbar spinal cord of SOD1-G93A transgenic mice. This study indicates that DATS has multifunctional neuroprotective effects in SOD1-G93A transgenic mice.

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive, adult-onset neurodegenerative disease that mainly affects motor neurons in cortex, brain stem, and spinal cord. SOD1 transgenic mice are the most widely used animal model of amyotrophic lateral sclerosis (Robertson et al., 2002). Mice carrying the human G93A mutation (SOD1-G93A) develop a phenotype similar to that of ALS patients (Gurney et al., 1994; Ripps et al., 1995; Tu et al., 1996). The mechanism of motor neuron degeneration in ALS remains to be elucidated. Several hypotheses have been proposed including glutamate-induced excitotoxicity, oxidative stress, cytoskeletal abnormality, and protein aggregation (Rothstein, 1995; Carrí et al., 2003; Julien, 1995; Prudencio et al., 2009). Although many compounds targeting one or more of these mechanisms have been evaluated clinically, riluzole was the only drug found to be effective in preventing disease progression (Costa et al., 2010).

Diallyl trisulfide (DATS) is one of the major organosulfur compounds (OSCs) in garlic oil (GO). OSCs, known as a group of potential chemopreventive compounds, can modulate phase II detoxifying enzymes. DATS activates Nrf2, causes ARE activation, and induces phase II gene expression in human hepatoma HepG2 cells (Chen et al., 2004). DATS can reduce LPS-induced iNOS expression, NO production, and NF-kappaB activation in RAW 264.7 macrophages (Liu et al., 2006). In our
previous experiment, DATS protected motor neurons against glutamate-induced excitotoxicity by activating Nrf2 and inducing expression of phase II enzyme, NQO1 (Sun et al., 2009). Based on these studies, we decided to examine the neuroprotective effect of DATS in preventing disease progression in ALS mouse model.

2. Results

2.1. DATS treatment prolongs disease duration and survival in SOD1-G93A transgenic mice

Time of disease onset was determined as the time when body weight declined over 2 successive recordings, failure of hind limb splaying when suspended by grabbing the tail, gait abnormalities, or short time to remain on the rod (less than 90 sec) (Kieran et al., 2004; Lobsiger et al., 2005). Treatment with DATS or corn oil (vehicle) was initiated at disease onset stage of 105.6±5.4 and 105.9±5.1 days of age, respectively (Table 1). No significant difference was observed between groups at the beginning age of treatment. DATS treatment caused an extended life span compared with vehicle-treated SOD1-G93A mice. DATS-treated group showed significantly prolonged disease progression (Fig. 1). The decline of muscle strength and motor performance was clearly delayed. Disease duration of DATS-treated group was prolonged by 9.1 days (1.3 weeks), i.e., from 36±11.0 days in vehicle-treated mice to 45.1±8.4 days in DATS-treated mice. Life span was prolonged by 7.7 days (1.1 weeks), from 142.4±10.2 days in vehicle-treated mice to 150.1±9.5 days in DATS-treated mice (Table 1).

2.2. Comparison of clinical manifestation and pathological changes between mice treated with DATS and vehicle

At the beginning of treatment, no obvious difference in step length was observed between vehicle- and DATS-treated groups, showing 3.06±0.25 cm and 3.18±0.20 cm for fore limb, 3.00±0.23 cm and 3.20±0.15 cm for hind limb, respectively. After 20 days treatment which began at disease onset, DATS-treated SOD1-G93A mice showed longer step length than vehicle-treated mice. The step length of fore limb was 1.03±0.62 cm in vehicle-treated mice compared with 2.59±0.38 cm in DATS-treated mice. In addition, the step length of hind limb was 1.04±0.71 cm in vehicle-treated mice compared with 2.75±0.31 cm in DATS-treated mice (Fig. 2). Pathologically, DATS-treated mice showed less vacuoles and inclusions and relatively more preserved motor neurons in the lumbar anterior horn compared with vehicle-treated mice after 20 days of treatment (Fig. 3).

2.3. DATS induces heme oxygenase-1 expression and reduces astrocytic activation

Heme oxygenase-1 (HO-1) is the inducible isoform that catalyzes the first and rate-limiting heme degradation. HO-1 not only protects against oxidative stress but possesses anti-inflammatory functions (Vijayan et al., 2010). Astrocytic proliferation is one of the most common pathological changes in the lumbar spinal cord of SOD1-G93A transgenic mice. Glial fibril acid protein (GFAP) is the specific intermediate filament of astrocytes. Therefore, we performed Western blot analysis to examine the HO-1 and GFAP expression at the end stage of SOD1-G93A mice treated with and without DATS. HO-1 expression was induced in vehicle-treated SOD1-G93A mice compared with non-transgenic littermates, while DATS-treated SOD1-G93A mice showed increased HO-1 expression induction compared with vehicle-treated mice. A clear increase of GFAP expression was detected in vehicle-treated SOD1-G93A mice compared with non-transgenic littermates, while GFAP increase was significantly reduced in DATS-treated SOD1-G93A mice (Fig. 4).

3. Discussion

ALS is a devastating neurodegenerative disorder characterized by selective motor neuron death, the causes of which are not completely understood. Substantial evidence implicates that oxidative stress play a key role in motor neuron injury and some antioxidants has been tried as potential therapies for ALS (Barber and Shaw, 2010). DATS, one of the principal constituents of GO, has been reported to have anticancer effects and cause induction of phase II detoxification enzymes in experimental liver injury (Seki et al., 2008; Table 1 – Comparison of disease duration and life span between vehicle- and DATS-treated SOD1-G93A transgenic mice.

<table>
<thead>
<tr>
<th>Number of mice</th>
<th>Age of treatment</th>
<th>Disease duration</th>
<th>Life span</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (14)</td>
<td>105.9±5.1</td>
<td>36.0±11.0</td>
<td>142.4±10.2</td>
</tr>
<tr>
<td>DATS (15)</td>
<td>105.6±5.4</td>
<td>45.1±8.4</td>
<td>150.1±9.5</td>
</tr>
</tbody>
</table>

DATS-treated SOD1-G93A mice demonstrated extended disease duration and life span compared with vehicle-treated mice. The values are means±SD.

* P<0.05.
Fukao et al., 2004). We have previously showed the neuroprotective effects of DATS in glutamate-mediated motor neuron injury in vitro (Sun et al., 2009). Herein, in vivo study was performed to test whether DATS could prolong the survival of SOD1-G93A transgenic mice. A previous study revealed that oral gavage of 2 mg DATS (approximately 80 mg/kg), thrice per week, was well-tolerated by mice (Singh et al., 2008), which was also used in the present study.

In this study, we observed that DATS treatment that began at disease onset prolonged disease duration and survival for about one week, compared with vehicle-treated SOD1-G93A mice. DATS treatment improved motor performance and ameliorated pathological changes, including reduced vacuoles and preserved motor neurons. To our knowledge, this is the first study to examine the neuroprotective effects of DATS in ALS transgenic mouse model.

HO-1 is the rate-limiting enzyme converting heme to biliverdin, free iron, and carbon monoxide (CO). HO-1 was rapidly upregulated under various stress conditions and has potent physiological regulating properties. HO-1 induction led to enhanced resistance to various stresses (Tuzuner et al., 2004; Yao et al., 2009). All of the products of heme catalysis, biliverdin/bilirubin (Berberat et al., 2005), CO (Ryter and Choi, in press), and iron (that induces apoferritin), have been reported to mediate the beneficial effects of HO-1. Recently, HO-1 has been recognized to have immunomodulatory and anti-inflammatory properties. Moreover, inducible HO-1 gene expression was controlled by redox-dependent transcriptional activators including Nrf2, NF-kB, and AP-1 (Paine et al., 2010). In this study, we observed a significant HO-1 induction in the lumbar spinal cord in DATS-treated SOD1-G93A mice, compared with vehicle-treated SOD1-G93A mice. The result indicates that HO-1 may contribute to the neuroprotective effects of DATS.

Another explanation for the neuroprotective effects of DATS lies in the inhibition of astrocytic activation. Our previous studies showed that GFAP expression increased progressively with the disease progression in the lumbar spinal cord of SOD1-G93A transgenic mice (Guo et al., 2010). Currently, increasing evidences support that reactive astrogliosis plays a potential toxic role in shaping motor neuron survival in ALS (Vargas and Johnson, 2010). Therefore, compounds targeting at reducing astrocytic activation may be beneficial for treatment of ALS. Our studies demonstrate that GFAP expression was reduced in DATS-treated mice, compared with vehicle-treated mice.
In summary, DATS extends survival of SOD1-G93A transgenic mice, induces HO-1, and reduces astrocytic activation. DATS is a promising neuroprotective agent against ALS.

4. Experimental procedures

4.1. Animals

Transgenic human SOD1-G93A mice and their non-transgenic littermates were generated by breeding male hemizygous carriers (B6SJL-Tg(SOD1-G93A)1Gur/J) to female B6SJL-F1 hybrids, both of which were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). PCR-based genotyping of tail/blood DNA was used to identify the transgenic mice (Gurney et al., 1994). The animals were maintained in a 12 h light/dark cycle with ad libitum access to water and diet. Experiments were carried out according to the regulations of laboratory animal management promulgated by the Ministry of Science and Technology of the People’s Republic of China, which are in accordance with the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals. For humane reasons, the animals were considered as end stage and sacrificed when they were unable to right themselves within 20 s after placed on either side or its back.

4.2. Treatment of DATS

Thirty-nine female SOD1-G93A transgenic mice were randomly divided into DATS-treated group and vehicle-treated group. DATS and vehicle treatment were given once a day from the disease onset stage. Mice (Tg mice, n=20; non-Tg littermates, n=4) were orally administered DATS at 80 mg/kg body weight/day (LKT Laboratories, St. Paul, MN, dissolved in corn oil) or an equal volume of corn oil (Tg mice, n=19; non-Tg littermates, n=4).

4.3. Clinical assessment

To monitor disease progression, the animals were subjected to tail suspension test daily and weighed twice a week from 12 weeks of age. When the earliest gait abnormalities were presented or two successive weight losses were recorded or when an animal could not remain on the rotating cylinder (d=30 mm) of a Rota-Rod apparatus at a constant speed of 30 rpm (YLS-4C Rota-Rod, Shandong Academy of Medical Sciences) for 90 s, it was concluded that disease onset was reached and treatment was started. Footprints were recorded in some of the mice (5 DATS-treated and 5 vehicle-treated SOD1-G93A mice) and sacrificed for pathological examination after 20 days treatment. The remaining animals were treated continuously to the end stage.

Fig. 3 – Representative micrographs of lumbar anterior horn showing relatively reserved motor neurons and reduced vacuoles in DATS-treated SOD1-G93A mice compared with vehicle-treated SOD1-G93A mice. C and D are the expanded areas framed in dashed lines in A and B, respectively. Arrows indicate inclusions and arrowheads indicate vacuoles. Bar = 100 μm
4.4. Histopathological examination

Mice were anesthetized with 10% chloral hydrate (0.2 ml/mice) and perfused transcardially with 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4. The spinal cords were carefully dissected. The lumbar enlargements (L3–5) were fixed in 4% paraformaldehyde for further 24 h and then grade-dehydrated with ethanol, embedded in paraffin, and sliced to 5 μm sections. The sections were then deparaffinized, hydrated, and stained with hematoxylin and eosin (H&E). After dehydration, the sections were mounted with neutral gum and observed with a Nikon 50i microscope.

4.5. Immunoblotting analysis

Whole tissue extracts were prepared using a total protein extraction kit (Applygen Technologies Inc.) following the manufacturer’s instruction. Protein levels in the extracts were quantified using the Bradford method. Forty micrograms of protein from each sample was run on 10% SDS-PAGE gels and blotted onto PVDF membranes. After probing the membranes with specific antibodies, including anti-β-actin, anti-GAPDH, and anti-SOD1 (Santa Cruz, CA, USA), anti-HO-1 (Stressgen Biotechnologies, Victoria, Canada) and anti-GFAP (Chemicon, Temecula, CA), IRDye™ 700DX anti-mouse IgG, IRDye™ 800DX anti-rabbit IgG, and IRDye™ 800DX anti-goat IgG (Rockland, Maine, USA) were used and the bands of interest were detected using an Odyssey Infrared Imaging System (LI-COR, Lincoln, NE). Band intensity was quantified using the Odyssey Infrared Imaging System Version 2.1.12 and was normalized by the β-actin or GAPDH band. The original green or red color of a band was converted to black and white colors for data presentation.

4.6. Statistical analysis

Results are expressed as means±SD. Statistical analyses were performed using one-way ANOVA followed by SNK multiple-range test with SAS 8.0 statistical software. Differences were considered significant at P<0.05.

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