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Neuroprotective effect of oxidized galectin-1 in a transgenic mouse model of amyotrophic lateral sclerosis

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Abstract

Abnormal accumulation of neurofilaments in motor neurons is a characteristic pathological finding in amyotrophic lateral sclerosis (ALS). Recently, we revealed that galectin-1, whose oxidized form has axonal regeneration-enhancing activity, accumulates in the neurofilamentous lesions in ALS. To investigate whether oxidized galectin-1 has a beneficial effect on ALS, oxidized recombinant human galectin-1 (rhGAL-1/ox) or physiological saline was injected into the left gastrocnemius muscle of the transgenic mice over-expressing a mutant copper/zinc superoxide dismutase (SOD1) with a substitution of histidine to arginine at position 46 (H46R SOD1). The H46R SOD1 transgenic mice, which represented a new animal model of familial ALS, were subsequently assessed for their disease onset, life span, duration of illness, and motor function. Furthermore, the number of remaining large anterior horn cells of spinal cords was also compared between the two groups. The results showed that administration of rhGAL-1/ox to the mice delayed the onset of their disease and prolonged the life of the mice and the duration of their illness. Motor function, as evaluated by a Rotarod performance, was improved in rhGAL-1/ox-treated mice. Significantly more anterior horn neurons of the lumbar and cervical cords were preserved in the mice injected with rhGAL-1/ox than in those injected with physiological saline. The study suggests that rhGAL-1/ox administration could be a new therapeutic strategy for ALS.

Keywords: Amyotrophic lateral sclerosis; Oxidized galectin-1; Cu/Zn superoxide dismutase; Transgenic mice

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by loss of motor neurons in the cerebral motor cortex, brainstem, and spinal cord. ALS shows progressive muscle weakness and atrophy, with most patients dying within 5 years of disease onset (Cleveland, 1999; Rowland and Schneider, 2001). Usually, ALS occurs sporadically; however, approximately 10% of these patients with familial ALS (FALS), 10–20% have missense mutations or a small deletion of the gene encoding Cu/Zn superoxide dismutase (SOD1) (Cleveland, 1999; Rowland and Schneider, 2001). Several lines of transgenic (Tg) mice with a FALS-linked mutated SOD1 gene have been made, and these mice have developed an adult onset paralytic disorder that is similar to sporadic and familial ALS (Bruijn et al., 1998; Gurney et al., 1994; Ripps et al., 1995; Tu et al., 1996). Accordingly, those Tg mice with SOD1 mutations have been used as an animal model for ALS.

Immunohistochemical investigations of the ALS spinal cord have shown that an abnormal accumulation of neuro-

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filaments in the cytoplasm and cell processes is a common pathological hallmark of both sporadic and familial ALS (Cleveland, 1999). The abnormal accumulation of neurofilaments induces axonal spheroids, cord-like neurite swellings, and perikaryal conglomerate inclusions in degenerating motor neurons of the spinal cord. These pathological features are considered to be important early pathological changes in ALS (Hirano et al., 1984; Kato et al., 2001). Therefore, an investigation of the accumulation of neurofilaments may help to understand the pathogenesis of motor neuron degeneration in ALS.

We have previously reported that galectin-1, which is a member of the β (beta)-galactoside-binding lectins, is accumulated in neurofilamentous lesions of the spinal cord in both sporadic and familial ALS (Kato et al., 2001). Galectin-1 has been shown to take two molecular forms: oxidized and reduced (Inagaki et al., 2000). Since oxidized galectin-1 has been reported to promote axonal regeneration after a peripheral nerve injury (Fukaya et al., 2003; Horie and Kadoya, 2000; Horie et al., 1999; Inagaki et al., 2000), it is possible that oxidized galectin-1 promotes the survival of the degenerating motor neurons in ALS.

The Tg mice expressing mutant human SOD1 with a substitution of histidine to arginine at position 46 (H46R SOD1) were established as a new animal model of familial ALS. In the present investigation, we administered oxidized galectin-1 to the H46R SOD1 Tg mice and subsequently assessed their disease onset, life span, duration of illness, and motor function. The present study showed that oxidized galectin-1 had a beneficial effect on the motor function and survival of Tg mice. This is the first report of a possible therapeutic effect of oxidized galectin-1 on ALS.

Materials and methods

Construction of transgenic (Tg) mice expressing mutant human SOD1

The present study was performed on Tg mice expressing mutant human SOD1 with a substitution of histidine to arginine at position 46 (H46R SOD1). We isolated a clone containing the full genomic human SOD1 gene, which was identified by screening a human genomic PAC library (Ioannou et al., 1994) using PCR with pairs specific to the human SOD1 gene. Subsequently, we cloned an 11.5 kb *Eco*RI–*Bam*HI fragment that contained the entire coding sequence and promoter region of the human SOD1 gene (Elroy-Stein et al., 1986; Levanon et al., 1985). The H46R mutation was engineered into this fragment by site-directed mutagenesis (Mutan-express Km, Takara, Otsu, Japan). The mutagenic primer and selection primer, which restored the Km resistance, hybridized to the vector and were incorporated during replication. The resulting potential Km resistant clone was subsequently sequenced (oligonucleotide-directed dual amber method) (Hashimoto-Gotoh et al., 1995) to verify the presence of either of the introduced mutations.

A linear 11.5 kb *Eco*RI–*Bam*HI fragment containing the H46R mutation was microinjected into BDF1 (C57BL/6 × DBA/2 F1) mouse (Jackson Laboratories, Bar Harbor, ME) embryos. The treated embryos were subsequently transferred to the oviducts of pseudopregnant ICR-scl female mice. The male littermates that were heterozygous for the H46R SOD1 mutation were used in this study. The mutated H46R SOD1 gene was identified by tail-clip PCR amplification using human SOD1-specific primers (sense primer: 5′-TTGGGAGGAGGTAGTGATTA-3′ and anti-sense primer: 5′-AGCTAGCAGGATAACAGATGA-3′). PCR was conducted with 1 cycle at 94°C for 2 min followed by 25 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s. Founder mice were then mated with C57B/6 mice (Jackson Laboratories).

Histopathological and immunohistochemical analysis

The mice were anesthetized with diethyl ether and killed by transcardiac perfusion with physiological saline followed by 4% paraformaldehyde containing phosphate-buffered saline (PBS; pH 7.4). The spinal cord was removed, post-fixed in the above solution, and embedded in paraffin. Serial transverse sections (4 μm thickness) of the lumbar segment ($L_{4–5}$) were cut and stained with hematoxylin and eosin (H&E) for a routine histological investigation. Several sections were also used for immunohistochemical investigations. Immunohistochemistry was performed using antibodies against human SOD1 (dilution 1:500, MBL, Japan), ubiquitin (dilution 1:100, DAKO, Denmark), and glial fibrillary acidic protein (GFAP; dilution 1:500, DAKO, Denmark). Deparaffinized sections were incubated with 1% H_2O_2 in distilled water for 10 min followed by 5% normal goat serum. The sections were subsequently incubated with primary antibodies in PBS containing 0.03% Triton X-100 at 4°C for 48 h. They were then incubated with a biotinylated secondary antibody (Vector, Burlingame, CA) for 2 h. After incubation with the avidin–biotin–peroxidase complex (ABC; Vector) for 1 h, peroxidase labeling was visualized by incubating the sections with 0.05 M Tris-buffered saline containing 0.05% 3,3′-diaminobenzidine tetrahydrochloride (DAB), 0.05 M imidazole, and 0.00015% H_2O_2 to yield a brown reaction product. The sections were then counter-stained with hematoxylin. Non-Tg mice were used for a comparison of histological findings.

Preparation of oxidized recombinant human galectin-1 (rhGAL-1/ox)

rhGAL-1/ox was obtained according to previous methods (Inagaki et al., 2000). In brief, rhGAL-1 was expressed in *Escherichia coli* and purified from the supernatant of the sonicated *E. coli* by DEAE-HPLC. Oxidized galectin-1

was obtained from bacterially expressed rhGAL-1 by the air oxidation method with $CuSO_4$ as a catalyst. DEAE-purified rhGAL-1 was subsequently diluted 20-fold with 20 mM Tris–HCl, pH 8.0, $CuSO_4$ was added to a final concentration of 0.0001% (w/v), and the mixture was maintained overnight at 4°C to allow disulfide bound formation.

rhGAL-1/ox was then purified by reversed-phase HPLC on a YMC-pack Protein RP column (YMC) with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid. The purified rhGAL-1/ox contains three intramolecular disulfide linkages between Cys^2 and Cys^{130}, Cys^{16} and Cys^{88}, and Cys^{42} and Cys^{60}, which represent a stable conformation of oxidized galectin-1. Analysis by SDS-PAGE and HPLC revealed that rhGAL-1/ox was not degenerated even after 10 days of incubation at 37°C in PBS (5 μl protein/ml). rhGAL-1/ox confirmed that the protein promotes axonal regeneration in both the in vitro examination (Horie et al., 2004) and in the in vivo acellular nerve regeneration model (Fukaya et al., 2003).

Experimental protocol

Kadoya et al. recently reported that the application of rhGAL-1/ox (0.125 μg/body weight (g)/week) to the injured region promotes the restoration of nerve function using in vivo peripheral nerve regeneration model (Kadoya et al., in press). No one showed any toxic effects in reaction to the administration of rhGAL-1/ox. The concentration of rhGAL-1/ox in the present study was chosen because previous investigation demonstrated the effect and safety in an animal model and because a higher dose would be expected to promote the survival of the degenerating motor neurons. For the reasons above, 0.25 μg/g (body weight)/week of rhGAL-1/ox was administered to the H46R SOD1 Tg mice. Furthermore, intramuscular administration of rhGAL-1/ox was performed on a weekly basis in considering the application of rhGAL-1/ox to human ALS in the future.

The Tg mouse littermates were randomly divided into two groups: (1) administration of rhGAL-1/ox of 0.25 μg/g (body weight) (gal-1-treated group); and (2) administration of 60 μl physiological saline (control group). rhGAL-1/ox was diluted with physiological saline and injected into the left gastrocnemius muscle using a microsyringe connected to a 27-gauge needle. The control group received physiological saline instead of rhGAL-1/ox. To clarify whether rhGAL-1/ox delays the onset of the disease, intramuscular injection was started prior to the early presymptomatic stage. At postnatal day 70 (10 weeks of age), the mice started receiving a weekly intramuscular injection of rhGAL-1/ox or physiological saline. Twenty-eight transgenic mice (gal-1-treated group, $n = 14$; control group, $n = 14$) were used for the assessment of disease onset, life span, and duration of illness. Among them, 19 transgenic mice (gal-1-treated group, $n = 10$; control group, $n = 9$) were used for the assessment of motor function. Additional transgenic mice (gal-1-treated group, $n = 6$; control group, $n = 5$) were used for the histological and pathological investigation.

Assessment of motor function

Motor function was assessed using a Rotarod (Muromachi Instruments, Tokyo, Japan) on a weekly basis. The period for which a mouse could remain on a rotating axle (diameter, 30 mm; two sets of rotation speed, 5 and 20 rpm) without falling was measured. The time was automatically stopped if the mouse fell from the rod or after an arbitrary limit of 420 s (Li et al., 2000). Mice were tested once a week until they could no longer perform the task. An examiner who was blinded to the experimental design assessed the motor functions of the mice mentioned above. The onset of motor dysfunction was defined as the first day when a mouse could not remain on the Rotarod for 420 s at a speed of 20 rpm, as described previously (Li et al., 2000). The life span was defined as the postnatal day when the mouse died (Shefner et al., 2001). The duration of illness was defined as the number of days from the onset of motor dysfunction to death (Wang et al., 2002).

Assessment of body weights with transgenic mice

The body weight of each transgenic mouse was assessed on a weekly basis. Twenty-eight transgenic mice (gal-1-treated group, $n = 14$; control group, $n = 14$), which were used for the assessment of survival, were also used for the assessment of body weights.

Histopathological examination: tissue preparation and cell counting

The mice, which had been anesthetized with diethyl ether, were sacrificed by transcardiac perfusion with physiological saline followed by 4% paraformaldehyde containing PBS (pH 7.4). The spinal cord was removed, post-fixed in the above solution, and embedded in paraffin. Serial transverse sections (4 μm thickness) of the lumbar segment ($L_{4–5}$) were cut and stained with H&E for a routine histological investigation.

The number of spinal anterior horn neurons was examined at postnatal day 147 (21 weeks of age). Thirty serial sections each (10 μm thickness) of the cervical ($C_{5–6}$) and the lumbar cords ($L_{4–5}$) were stained by the Nissl method and photographed under light microscopy at 40× magnification. An examiner who was blinded to the experimental design counted the anterior horn cells that met all of the following criteria: (1) neurons located in the anterior horn ventral to the line tangential to the ventral tip of the central canal; (2) neurons with a maximum diameter of 20 μm or more; and (3) neurons with a distinct nucleolus (Manabe et al., 2003; Warita et al., 1999).

Statistical analysis

For the two groups, the disease onset, length of survival, and duration of illness were evaluated using the Log-rank. The results of motor function tested with the Rotarod and the body weights of transgenic mice were statistically analyzed using ANOVA for multiple comparisons among the groups. The number of anterior horn neurons in each group was compared using the two-tailed Student's t test. Statistical analysis was performed using computerized software (SPSS ver. 11.0, Chicago, Illinois, USA). $P < 0.05$ was accepted as statistically significant.

The experiment was approved by the Committee on Ethics of Animal Experiments and followed the Yamagata University School of Medicine Guidelines for Animal Experiments.

Results

Clinical phenotype and course of the transgenic mice

All the mice used had H46R mutant SOD1, as demonstrated by tail-clip PCR. These mice presented motor dysfunction similar to human ALS; the first sign of disease was weakness in their hind limbs, mostly shown by dragging of one limb. As the disease progressed, the mice showed marked muscle wasting in their limbs. The other muscles also became weak; thereafter, the affected mice could not move to reach their water supply and died. The motor dysfunction occurred at about 20 weeks of age, and most mice died at about 24 weeks of age.

Histopathological and immunohistochemical studies in the spinal cords

In mice at the early presymptomatic stage (91 days of age), no apparent changes were observed in the H&E-stained sections (Fig. 1A). Small numbers of GFAP-immunoreactive astrocytes were seen (Fig. 1E). At the late presymptomatic stage (119 days of age), large anterior horn cells seemed to decrease in number (Fig. 1B). By 147 days of age (the early symptomatic stage), when weakness of the limbs became apparent, there was a marked loss in the number of anterior horn cells (Fig. 1C) accompanied by astrocytic proliferation (Fig. 1G). Neurite swellings in the anterior horns were also observed (Fig. 2B). Eosinophilic inclusion bodies similar to Lewy body-like hyaline inclusions in human ALS were detectable in the anterior horns. These inclusions were immunostained with anti-SOD1 antibody and anti-ubiquitin antibody, most of which were detected in the neuropil (Figs. 2A and C), and only a few were within the perikarya of neurons (Fig. 2D). At the end stage, there was a severe decrease in the number of anterior horn neurons (Fig. 1D) with diffuse astrocytic proliferation (Fig. 1H). There were no remarkable vacuoles like those in cell bodies, dendrites, and axons of previously reported transgenic mice expressing SOD1 mutation G37R (Wong et al., 1995) or G93A (Gurney et al., 1994).

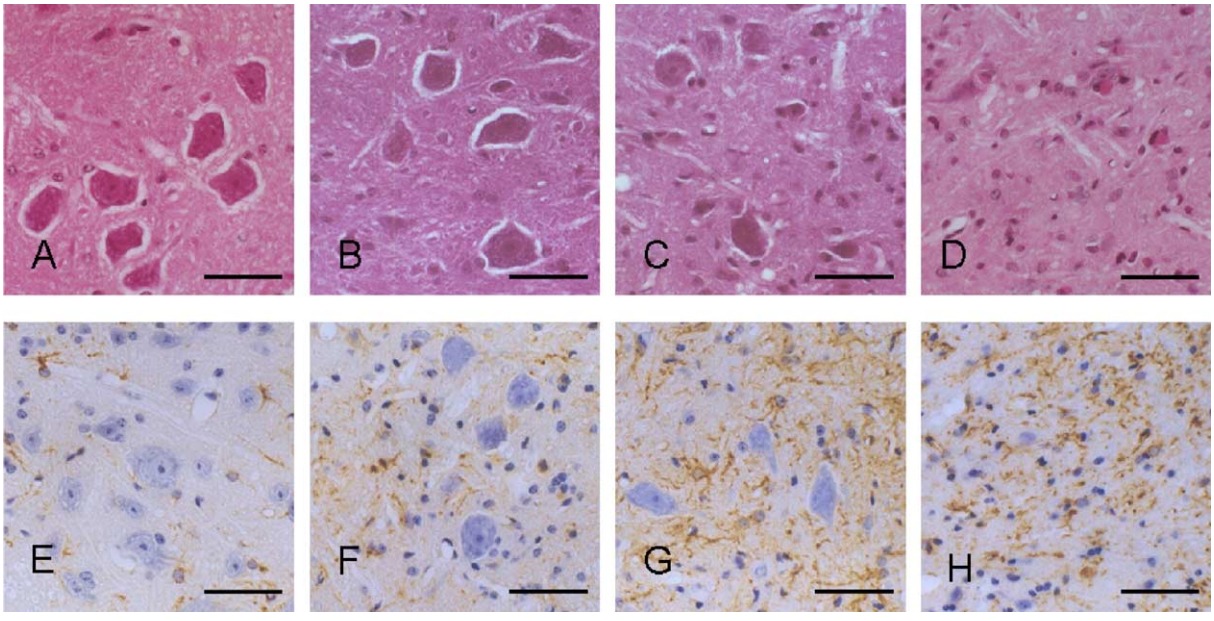

Fig. 1. Histopathological findings of the lumbar cord in the H46R transgenic mice. Sections were stained with hematoxylin–eosin (H&E) (A–D) and immunostained with anti-glial fibrillary acidic protein (GFAP) antibody (E–H). (A, E) No apparent changes are observed in the section stained with H&E in the early presymptomatic stage (91 days of age). Small numbers of GFAP-immunoreactive astrocytes are seen. (B, F) The number of large anterior horn cells seems to decrease in the late presymptomatic stage (119 days of age) accompanied by astrocytic proliferation. (C, G) A marked loss of anterior horn cells is seen with an increase in the number of astrocytes at the early symptomatic stage (147 days of age). (D, H) A severe loss of anterior horn neurons is observed at the end stage (168 days of age). Scale bars = 50 μm.

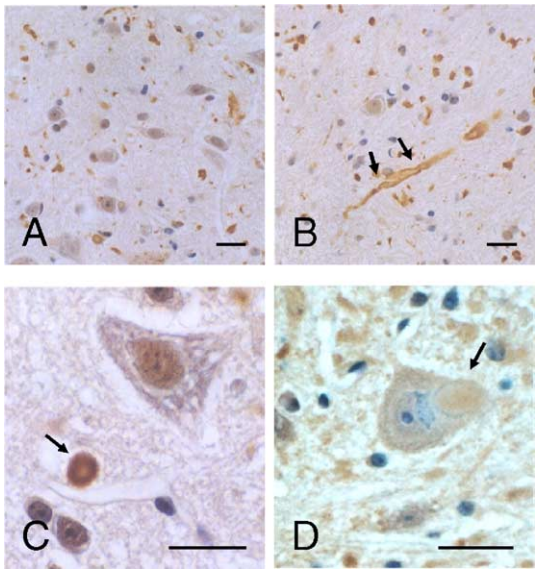

Fig. 2. (A) Many inclusion bodies, immunostained with anti-ubiquitin antibody, are readily apparent in the neuropil of the spinal ventral horn. (B) Cord-like neurite thickening of the ventral horn is immunostained with anti-ubiquitin antibody. (C) A Lewy body-like inclusion in neuropil is immunostained with anti-ubiquitin antibody. (D) A Lewy body-like inclusion immunostained with anti-SOD1 antibody is seen in the spinal anterior horn neuron. Scale bars = 20 μm.

Effects of rhGAL-1/ox on the disease course

rhGAL-1/ox delayed the onset of disease

The onset of the disease, as defined by falling from the Rotarod (20 rpm) within 420 s, occurred at postnatal day 138.3 ± 1.7 in the control group. The gal-1-treated mice showed a statistically significant delay of onset (postnatal day 143.5 ± 1.5) (P = 0.0156) (Fig. 3A and Table 1).

rhGAL-1/ox prolonged survival

The life span was much longer (172.2 ± 1.3 days) in the mice in the gal-1-treated group than in those in the control group (160.4 ± 2.4 days) (P < 0.0001) (Fig. 3B and Table 1).

rhGAL-1/ox prolonged the duration of illness

The duration of illness was significantly longer in the mice in the gal-1-treated group (28.7 ± 1.8 days) than in those in the control group (22.1 ± 1.4 days) (P = 0.0072) (Table 1).

rhGAL-1/ox improved motor function

In the condition with 5 rpm, which was a weak task, differences in motor function between the two groups were not apparent at postnatal day 133 (19 weeks of age). Although motor function appeared to be better in the mice in the gal-1-treated group than in those in the control group at the early symptomatic stage, the difference between two groups was not statistically significant (ANOVA; P = 0.282) (Fig. 4A).

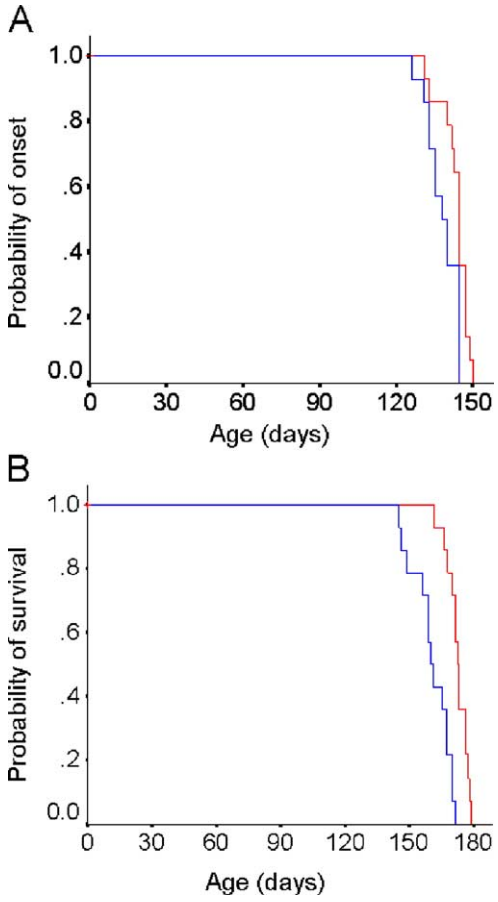

Fig. 3. rhGAL-1/ox delayed the disease onset and prolonged the survival of Tg mice (mutant H46R SOD1). The Kaplan–Meier curves demonstrate the probability of onset of Rotarod deficit (A) and length of survival (B) in Tg mice (mutant H46R SOD1). The onset of the Rotarod deficit was more delayed in the gal-1-treated group than it was in the control group (P = 0.0156) (A). The life span was significantly more prolonged in the gal-1-treated group than in the control group (P < 0.0001) (B). Red line, gal-1 group (n = 14); blue line, control group (n = 14).

In the condition with 20 rpm, which corresponded to a heavy task, motor function was better in the gal-1-treated mice than in the control group mice at postnatal day 103 (19 weeks of age). The motor function of the mice in the gal-1-treated group was significantly better than that in the mice in the control group (P = 0.038) (Fig. 4B).

The body weight changes of the transgenic mice

At the end stage (168 days of age), the mice in the control group showed a body weight loss by 7%, compared

Table 1
Onset of motor dysfunction, survival, and duration of illness

	Control (n = 14)	Gal-1 (n = 14)	P value
Onset (postnatal days)	138.3 ± 1.7	143.5 ± 1.5	0.0156
Survival (days)	160.4 ± 2.4	172.2 ± 1.3	<0.0001
Duration (days)	22.1 ± 1.4	28.7 ± 1.8	0.0072

Values tabulated are mean ± SEM. Statistical comparisons were with Log-rank test.

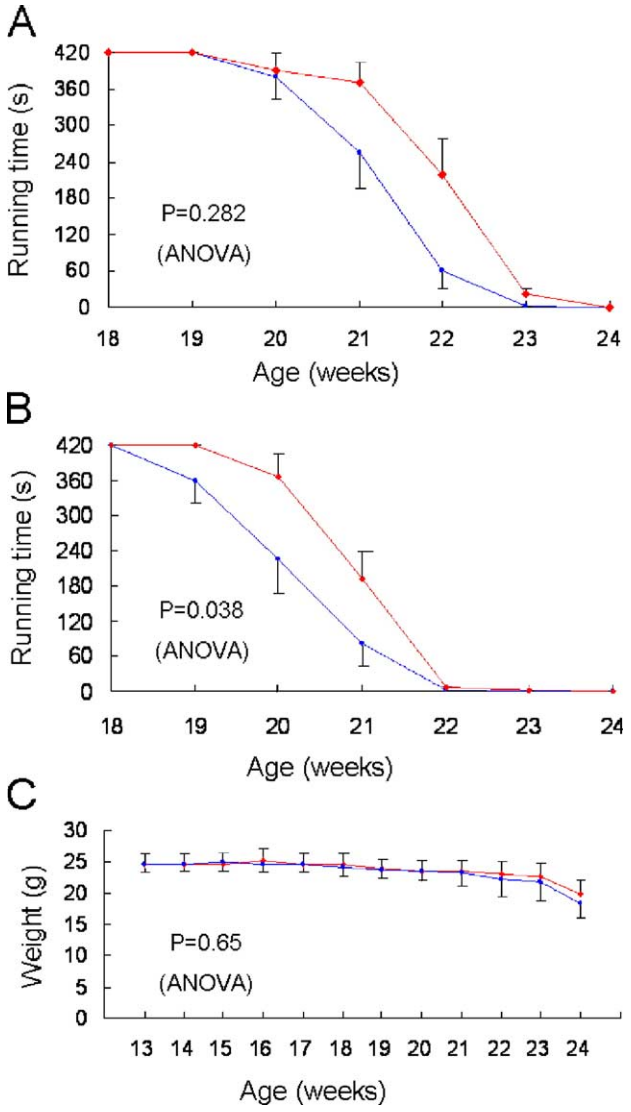

Fig. 4. Motor function of Tg mice (mutant H46R SOD1) tested with the Rotarod. For the 5 rpm task (A), there was no significant difference between the two groups. However, the assessment with the Rotarod task at 20 rpm was much more improved in the gal-1-treated group than in the control group ($P = 0.038$) (B). Red line, gal-1 group ($n = 10$); blue line, control group ($n = 9$). Body weight measurements of the transgenic mice treated with rhGAL-1/ox or physiological saline (C). Red line, gal-1 group ($n = 14$); blue line, control group ($n = 14$). Error bars represent SD.

with those in the gal-1-treated group (18.4 ± 2.4 versus 19.7 ± 2.3 g) (Fig. 4C); however, there was no statistical significance of the body weights between the two groups (ANOVA; $P = 0.65$).

Histopathological evaluation of spinal cords with 147-day-old mice: effect of rhGAL-1/ox on motor neuron survival

In H&E-stained sections, several pathological features were seen in both gal-1-treated group and control group. Neurite swellings, eosinophilic inclusion bodies similar to Lewy body-like hyaline inclusions in human ALS, and astrocytic proliferations were detectable in the anterior horns of the spinal cord. Large anterior horn cells were decreased in number in both groups, however, histological evaluation using Nissl-stained spinal cord sections of the 147-day-old mice suggested a neuroprotective effect of rhGAL-1/ox on spinal motor neuron survival.

In Nissl-stained sections, more anterior horn cells of $L_{4–5}$ segments were preserved in the gal-1-treated group (Fig. 5A) than in the control group (Fig. 5B) ($P = 0.007$, Table 2). Furthermore, we compared the number of remaining large anterior horn cells at the cervical level ($C_{5–6}$) between the gal-1-treated group and the control group. At the cervical level, gal-1-treated Tg mice also had a greater number of large anterior horn cells than the control group ($P = 0.039$, Table 2). In both the cervical and lumbar spinal cords, there was no significant difference in the number of anterior horn cells between the

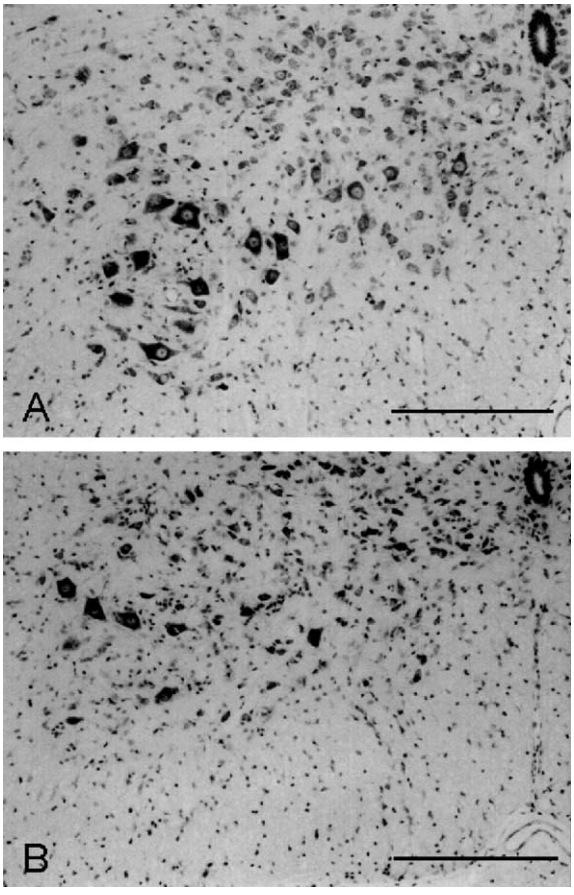

Fig. 5. Histological evaluation of the lumbar cord in 147-day-old mice. (A) rhGAL-1/ox-treated mice, (B) physiological saline-treated mice. In Nissl-stained sections, neuronal cells were well preserved in the anterior horn of the lumbar cord in the gal-1-treated group. Scale bars = 200 μm.

Table 2
The number of large anterior horn neurons/section of spinal cord $L_{4–5}$ and $C_{5–6}$ at postnatal day 147

	n	Lumbar cord		Total	Cervical cord		Total
		L	R		L	R	
Control group	5	3.8 ± 0.3	3.7 ± 0.3	7.5 ± 0.6*	5.7 ± 1.0	5.7 ± 1.1	11.4 ± 2.1*
Gal-1 group	6	6.9 ± 0.8	6.5 ± 0.7	13.5 ± 1.5*	10.0 ± 1.3	10.1 ± 1.6	20.1 ± 2.8*

Values tabulated are mean ± SEM. Statistical comparisons were with a two-tailed Student's *t* test.
n: number of mice examined; R: right side of the spinal cord; L: left side of the spinal cord.
* Gal-1 vs. Control, $P < 0.05$.

injected side (left) and the non-injected side (right) ($P > 0.05$, Table 2).

Discussion

The results of the present study showed the therapeutic effect of rhGAL-1/ox for H46R SOD1 Tg mice, an animal model of FALS. The administration of rhGAL-1/ox prevented the Tg mice from losing spinal anterior horn neurons. In contrast to the control group, rhGAL-1/ox-treated mice showed better behavioral performance and a prolonged life span, consistent with the preservation of spinal motor neurons. In the present study, rhGAL-1/ox was injected into the left gastrocnemius muscle. However, anterior horn cells were well preserved not only in the left side but also in the right side of the anterior horn of the lumbar cord. Moreover, the number of anterior horn cells was well preserved even in the cervical cord. Therefore, it seems that the effect of rhGAL-1/ox on the anterior horn cells is not through retrograde axonal transport.

Galectin-1, a member of the family of β-galactoside-binding lectins, is isolated as a homodimer of the 14.5 kDa subunit. Galectin-1 is present in various tissues and organs, including the lung, heart, skeletal muscle, skin, placenta, thymus, lymph node, brain, spinal cord, and peripheral nerve (Kasai and Hirabayashi, 1996). Several functions for galectin-1 have been proposed in those tissues: cell growth, cell differentiation, apoptosis, cell–cell interaction, and cell–matrix interaction (Perillo et al., 1998).

The galectin-1 molecule has six cysteine residues and, when it is oxidized, three disulfide bonds are formed (Inagaki et al., 2000). An oxidized form of galectin-1 showed axonal regeneration-enhancing activity; however, it lacked a property of lectin to bind to lactose (Inagaki et al., 2000). On the other hand, a reduce form of galectin-1 possessed lectin properties but showed no axonal regeneration-enhancing activity. Indeed, a galectin-1 mutant, in which all six cysteine residues were replaced by serine, induced lectin activity but lacked axonal regeneration-promoting activity (Inagaki et al., 2000).

These three intramolecular disulfide bonds appear to represent a stable conformation of oxidized galectin-1. As these strong covalent linkages are not broken down easily, injected rhGAL-1/ox probably acted as an oxidized form of galectin-1, showing axonal regeneration-enhancing activity. Indeed, rhGAL-1/ox confirmed that the protein promotes axonal regeneration in both in vitro experiments (Horie et al., 2004) and the in vivo acellular nerve regeneration model (Fukaya et al., 2003).

On the other hand, because direct application of oxidized galectin-1 to isolated primary sensory neurons does not alter their morphology, it is hypothesized that galectin-1 may stimulate non-neuronal cells to produce a factor that promotes Schwann cell migration while enhancing axonal regeneration (Horie et al., 1999, 2004). To date, the following issues have been addressed: (1) identification of target cells of galectin-1 among non-neuronal cells surrounding axons and/or neurons; (2) understanding of the mechanism whereby oxidized galectin-1 promotes axonal regeneration.

Recent reports have given possible answers to these questions. The macrophage is one target cell for oxidized galectin-1, and an axonal regeneration-promoting factor is secreted from macrophages stimulated by oxidized galectin-1 in vitro (Horie et al., 2004). Recently, Horie et al. have shown the following results: (1) macrophages bear specific receptors to rhGAL-1/ox on their cell membranes; (2) rhGAL-1 stimulates tyrosine phosphorylation of proteins in macrophages, suggesting that rhGAL-1/ox specifically binds to macrophages to activate their signal transduction pathway; (3) rhGAL-1/ox induces macrophages to secrete a factor(s) to promote axonal regeneration; (4) rhGAL-1/ox stimulates macrophages to enhance Schwann cell migration. Surprisingly, the axonal promoting activity of the conditioned medium secreted from galectin-1-activated macrophages is distinctively stronger than various trophic factors, such as nerve growth factor (NGF), insulin-like growth factor I (IGF-I), insulin-like growth factor II (IGF-II), and ciliary derived neurotrophic factor (CNTF) in vitro (Horie et al., 2004). Further experiments need to be conducted to identify the factor released from rhGAL-1/ox-stimulated macrophages.

To date, the mechanism of motor neuron degeneration in ALS remains unknown; however, several neurotrophic factors (NTFs) or other therapeutic agents have been studied because of their potential ability to protect against motor neuron degeneration. Indeed, these factors have been extensively studied in animal models of ALS. Several agents have shown delay of disease onset and/or survival prolongation, and these agents have been viewed as a new therapeutic strategy for ALS. As for these therapeutic agents,

the mechanisms of action have been considered to be as follows: (1) free radical scavengers (Barneoud and Curet, 1999; Dugan et al., 1997; Gurney et al., 1996); (2) glutamate inhibitors (Gurney et al., 1996); (3) copper chelator (Hottinger et al., 1997); (4) stabilizers of mitochondria (Klivenyi et al., 1999); (5) caspase inhibitors (Li et al., 2000); (6) microglial activation inhibitors (Kriz et al., 2002); and (7) NTFs. At present, riluzole, a glutamate receptor antagonist, is commercially available for patients with ALS (Rowland and Schneider, 2001). As for NTFs, some trials have been performed on patients with ALS; the subcutaneous delivery of IGF-I had marginal success in one of two human trials (Kaspar et al., 2003); however, other NTFs such as the CNTF, the glial cell line-derived neurotrophic factor (GDNF), and the brain-derived neurotrophic factor (BDNF) have been unsuccessful in human trials (Dawbarn and Allen, 2003).

Several investigations have revealed that the impairment of axonal transport is the early event of spinal motor neurons in ALS; disturbance of axonal transport may occur initially and subsequently cause accumulation of neurofilaments in the perikarya and the proximal portion of axons (Collard et al., 1995; Williamson and Cleveland, 1999; Zhang et al., 1997). Impairment of the axonal transport may trap galectin-1 in the perikarya and the proximal portion of the axons of the anterior horn cells in ALS (Kato et al., 2001). It has recently been reported that the axotomy of facial nerve induced transient upregulation of galectin-1 mRNA, suggesting that facial nerve injury can trigger the synthesis of galectin-1 in neuronal cell bodies (Akazawa et al., 2004). Several studies have also shown that galectin-1 is likely to be released from muscle cells and subsequently act as a factor for myogenesis in vivo (Goldring et al., 2002a,b; Gu et al., 1994).

If motor neuron axons and skeletal muscles truly need galectin-1 for their maintenance or survival, depletion of this protein may cause degeneration of the motor neurons and skeletal muscles. Although the mode of action of galectin-1 on spinal motor neurons remains unclear, the results of the present study show a potential therapeutic effect of galectin-1 for patients with ALS.

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