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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