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The lack of effect of specific overexpression of IGF-1 in the central nervous system or skeletal muscle on pathophysiology in the G93A SOD-1 mouse model of ALS

Maria Laura Messi¹, Heather M. Clark², David M. Prevette⁴, Ronald W. Oppenheim^{3,4}, and Osvaldo Delbono^{1,2,3,*}

¹Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157

²Department of Internal Medicine, Section on Gerontology, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157

³The Neuroscience Program, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157

⁴Department of Neurobiology and Anatomy, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157

Abstract

The ability of insulin like growth factor 1 (IGF-1) to prevent the pathophysiology associated with amyotrophic lateral sclerosis (ALS) is currently being explored with animal models and in clinical trials with patients. Several studies have reported positive effects of IGF-1 in reducing motor neuron death, delaying the onset of motor performance decline, and increasing life span, in SOD-1 mouse models of ALS and in one clinical trial. However, a second clinical trial produced no positive results raising questions about the therapeutic efficacy of IGF-1. To investigate the effect of specific and sustained IGF-1 expression in skeletal muscle or central nervous system on motor performance, life span, and motor neuron survival, human-IGF-1-transgenic mice were crossed with the G93A SOD-1 mutant model of ALS. No significant differences were found in onset of motor performance decline, life span, or motor neuron survival in the spinal cord, between SOD+/IGF-1+ and SOD+/IGF-1- hybrid mice. IGF-1 concentration levels, measured by radioimmunoassay, were found to be highly increased throughout life in the central nervous system (CNS) and skeletal muscle of IGF-1 transgenic hybrid mice. Additionally, increased CNS weight in SOD+ mice crossbred with CNS IGF-1 transgenic mice demonstrates that IGF-1 overexpression is biologically active even after the disease is fully developed. Taken together, these results raise questions concerning the therapeutic value of IGF-1 and indicate that further studies are needed to examine the relationship between methods of IGF-1 administration and its potential therapeutic value.

*Corresponding author: Osvaldo Delbono Department of General Internal Medicine, Gerontology Wake Forest University School of Medicine 1 Medical Center Boulevard, Winston-Salem, North Carolina 27157 Phone: 336-716-9802 Fax: 336-716-2273 E-mail: odelbono@wfubmc.edu

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Keywords

Amyotrophic Lateral Sclerosis; Insulin-like growth factor-1; motor neuron; cell death; skeletal muscle; transgenic; G93A SOD-1; motor performance

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease which is characterized by the selective loss of spinal, cranial, and cortical motor neurons and has a mean survival life-span of 3 to 5 years after diagnosis. Although the mechanism of ALS is still unclear, it is associated with oxidative stress, protein nitration/aggregation, and excitotoxicity (Martin et al., 2007). An estimated 10% of cases are familial and result from genetic mutations (Mulder et al., 1986). A superoxide dismutase-1 (SOD1) gene mutation has been linked to primary causes of disease in familial ALS (Rosen et al., 1993). The over-expression of G93A mutant human SOD-1 (SOD+) in mice is used as model for ALS (Gurney, 1994) (Julien and Kriz, 2006).

Insulin-like growth factor 1 (IGF-1) is a neurotrophic factor which crosses the blood brain barrier and is expressed in the central nervous system (CNS, brain, spinal cord) and skeletal muscle (Lewis et al., 1993). IGF-1's potential to enhance the outgrowth of spinal motor and corticospinal neurons makes it a prospective therapeutic agent in motor neuron disease (D'Costa et al., 1998) (Ozdinler and Macklis, 2006). Studies using adeno-associated viral (AAV) retrograde delivery of IGF-1 to mouse motor neurons *in vivo* shows that IGF-1 increases life-span and delays disease progression in SOD+ mice (Kaspar et al., 2003). Increased life-span, delayed disease progression, and enhanced motor neuron survival has also been reported in SOD+ mice crossed with transgenic mice over-expressing human IGF-1 in muscle (Dobrowolny et al., 2005).

While these studies in mouse models are encouraging, IGF-1 studies in humans have reported mixed results. In a double-blind, placebo controlled, randomized study supported by Cephalon, and Chiron, and the Muscular Dystrophy Association, the progression of functional impairment in patients receiving high doses (0.10mg/kg/day) of rIGF-1 was reduced by 26% versus patients receiving placebo (Lai et al., 1997). However, a double-blind study supported by Cephalon and Chiron using the same inclusion/exclusion criteria, screening period, and randomization criteria showed no significant difference between patients receiving rIGF-1 and placebo (Borasio et al., 1998). To help address these discrepant results, there currently is a phase III randomized, double-blind, placebo controlled clinical IGF-1 trial underway (clinicaltrials.gov, 2007).

Since human trials have shown varied results, we wanted to further examine the effect of IGF-1 treatment in the G93A ALS mouse model. Our approach of cross breeding SOD+ mice with transgenic mice over-expressing human IGF-1 (IGF-1) in brain or muscle should expose motor neurons to sustained and high IGF-1 concentrations throughout life. IGF-2/1 transgenic mice have high IGF-1 concentrations in the brain, cerebellum, and spinal cord (Ye et al., 1996) (Moreno et al., 2006), whereas S1/S2 transgenic mice express high levels of IGF-1 in skeletal muscle (Coleman et al., 1995; Messi and Delbono, 2003). By using both models, we have explored the effects of CNS-and-muscle derived IGF-1 on SOD-1+ mice. Additionally, we have examined the relationship between disease onset and life span with motor neuron size, motor neuron survival, and IGF-1 expression.

METHODS

IGF-1 transgenic and SOD1 mutant mice

In the present study, we used a transgenic mouse model (S1/S2) expressing human IGF-1 (IGF-1) exclusively in the skeletal muscle (Coleman et al., 1995; Renganathan et al., 1997; Renganathan et al., 1998). These mice were screened for the presence of hIGF-1 genomic DNA via excised mouse tail segments, which were digested overnight at 55 °C in digestion buffer containing 1M Tris-NaOH, pH 8.0, 5 M NaCl, 0.5 M ethylenediamine-tetraacetic acid, 20% sodium dodecyl sulfate and 20 mg/ml proteinase K. From this mixture, DNA was extracted by phenol:chloroform:isoamyl alcohol (25:24:1). IGF-1 gene from this DNA was screened by polymerase chain reaction (PCR) with specific 25-base primers: IGF-1 5': ATT TAA gTg Ctg CTT Ttg TgA TTT C and IGF-1 3' TTC CTA CAT CCT gTA gTT CTT gTT T. Amplified DNA fragments were analyzed for the presence of a clear 450-bp fragment specific for hIGF-1 on 2.5% agarose gels. Wild-type littermate FVB mice (S1/S2-) were used as controls.

The transgenic mice over-expressing hIGF-1 exclusively in the CNS (IGF-2/1 Tg), have been described previously (Ye et al., 1996). IGF2/1 is expressed in the CNS under the control of a 5.7 kb DNA fragment of the 5' mouse IGF-II genomic regulatory region (Dai et al., 1992; Ye et al., 1996). These IGF-1 mice have been backcrossed into a C57BL/6 background for more than 10 generations and are routinely bred as heterozygotes. The IGF2/1 transgene was identified by PCR analysis of genomic DNA using the following primers: 5' end primer: 5'-GGACCGGAGACGCTCTGCGG-3' (bp 179-198) and 3' end primer: 5'-CTGCGGTGGCATGTCACTCT-3' (complementary to bp 518-537).

Amplified DNA fragments were studied for the presence of a clear 360 bp fragment specific for brain IGF-1 on 3.0% agarose gels. Wild-type littermate C57BL/6 mice, named IGF2/1(-), were used as controls.

B6SJL-TgN(SOD1-G93A)1Gur (grey mice) and B6.Cg-Tg(SOD1-G93A)1Gur/J (black mice) were acquired from the Jackson laboratory (Bar Harbor, Maine). We will refer to these SOD mutant mice strains as gSOD(+) and bSOD(+), respectively. Both mice express a high copy number of the mutant allele human SOD1 containing the Gly93 to Ala (G93A) substitution; however, bSOD+ mice live longer than gSOD+ mice, 19-23 weeks compared to 16-20 weeks, and exhibit a more homogenous phenotype (Jackson laboratory, personal communication). This variation in phenotype may be related to different backgrounds. bSOD+ mice have a C57BL/6 background while gSOD+ mice have a B6SJL/F1 background. For detection of mutant human SOD-1, DNA was excised and isolated using the same procedure described for S1S2 transgenic mice except the primers used for the PCR were 5'-CAT CAg CCC TAA TCC ATC TgA-3' at 60 °C and 5'-CgC gAC TAA CAA TCA AA g TgA-3' at 59.0° C. The amplified 236bp DNA fragment representing mutant human SOD-1 was detected on 2.5% agarose gels.

For genomic DNA detection procedures in all mice, the interleukin gene was used as an internal control for proper DNA extraction and isolation. The PCR primers used for interleukin were 5'-CTA ggC CAC AgA ATT gAA AgA TCT-3' at 60°C and 5'-gTA ggT ggA AAT TCT AgC ATC ATC C-3' at 61.3 °C. Amplified DNA fragments were analyzed for the presence of a clear 324 bp fragment specific for interleukin on 2.5% agarose gels.

All mice were maintained in a 12:12h light:dark cycle at 22 °C. The colony was housed in a pathogen free facility of the Wake Forest University School of Medicine (WFUSM) Animal Resources Program. Mice exhibiting gross internal or external pathology at macroscopic inspection were not included in the study. Adequate measures were taken to minimize pain or discomfort and experiments were conducted in accordance with international and national

standards on animal welfare. All procedures were approved by the WFUSM Animal Care and Use Committee.

Cross-breeding of mice

Crossbreeding of gSOD and bSOD and their respective background littermates with either muscle (S1/S2) or brain (IGF2/1) IGF-1 transgenic over-expressing mice and their respective wild-type background littermates resulted in multiple combinations as described below. All SOD (+)/S1/S2(-) or SOD(+)/IGF2/1(-) and SOD(-) crossbred with S1/S2(+) or IGF2/1(+) hybrid mice were viable as demonstrated by survival beyond the 180 days reported in this work. The survival of wildtype mice was significantly shortened by crossbreeding with both SOD (+) mouse models as reported below.

IGF-1 concentration

IGF-1 concentration in serum, various regions of the CNS (brain, cerebellum, and spinal cord), soleus (SOL) and extensor digitorum longus (EDL) skeletal muscles, and other peripheral organs (heart and liver) was measured by radioimmunoassay (RIA) associated with IGF-1 binding protein blockade as described (Moreno et al., 2006). The RIA kit (Alpco Diagnostics, Windham, NH) used for these determinations, includes a specific, high affinity polyclonal antibody, which has a cross-reactivity with IGF-2 of less than 0.05%. The sensitivity of the assay is 0.02ng/ml.

Brain, cerebellum, brain stem, and spinal cord weights

IGF-1 overexpression results in organ enlargement (Ye et al., 1996). At age 6-7 weeks, mice were sacrificed and their brain, cerebellum, brain stem, and spinal cord blotted and weighed. Systematic anatomical references were used to separate the four CNS regions to make reliable and comparative weight determinations across and within mouse strains.

Motor Performance Test

Motor performance was assessed by the rotarod test using an Economex Rota-Rod with automatic fall detection (Columbus Instruments, Columbus, Ohio). Mice were placed on the rotarod apparatus revolving at either 10 and 18 rpm for a period of 120 seconds. Animals were tested for three trials and the longest duration achieved was computed and used for statistical analysis.

Life span

Survival time was measured by recording the date that each animal reached end-stage. Mice unable to right themselves after 30 seconds after being placed on their sides were defined as end-stage (Kaspar et al., 2003). This artificial time point allows mortality to be judged in a more humane and reliable fashion.

Motor Neuron Survival

Thionin-stained cryostat sections of spinal cord from mice were counted applying histological and morphometric procedures previously described (Li et al., 1994) (Clarke and Oppenheim, 1995) (Gould et al., 2006). The brain stem and spinal cord were cut transversely at 12-15 μ m. We counted all motor neurons in the ventral horn in every 10th section throughout the entire lumbar spinal cord. Only those cells with substantial cytoplasm, a nucleus, and containing at least one nucleolus were counted (Clarke and Oppenheim, 1995). Using these rather stringent criteria for cell counting, less than 1% of the cells appear on two successive sections and, therefore, only an insignificant number of cells are counted twice. The counts presented are the raw or actual cell counts multiplied by 10 and then divided by the total number of sections

per region to yield cells per section. Because motor neuron atrophy occurs in SOD1 mice, we included both large ($>300\mu\text{m}^2$) and small ($<300\mu\text{m}^2$) neurons within the ventral horn of mice from all genotypes. Even the smallest neurons in the ventral horn were distinct from non-neuronal cells (Fig. 1).

Soma Size

Thionin-stained somas were traced using a camera lucida, scanned, and cross-section areas measured using Scion (Frederick, MD) Image software (Buss et al., 2006). Because cross-sectional area underestimated cell size changes, we used cell volume for all soma size calculations (Buss et al., 2006). Only neurons with a distinct cell membrane and clear nucleus were included in these measurements.

Data Analysis and Statistics

All data are presented as mean $\pm$ s.e.m. Data were analyzed with Student's t-test, twoway repeated measures ANOVA, or with a Mann–Whitney rank sum test where appropriate. *P* values less than 0.01 (**) or less than 0.05 (*) were considered significant. *P* values greater than 0.05 were considered not significant (NS).

RESULTS

Motor performance

To determine IGF-1's effect on the clinical course of ALS, we conducted motor performance tests using the rotarod. For this analysis, gSOD+ /S1S2- and gSOD+ /S1S2+ mice and controls, gSOD- /S1S2- and gSOD- /S1S2+ mice, were tested at 10 and 18 rpm. Starting at day 107, we recorded the probability to fall in seconds with 120 seconds being the maximum value. We used day 107 as a starting point because it has been reported to be the approximate time of clinical onset for the G93A SOD+ mouse model (Kaspar et al., 2003) (Matsumoto et al., 2006). In both gSOD+ /S1S2- mice and gSOD+ /S1S2, there was a marked decrease in motor performance at day 107 at both 10 and 18 rpm compared with gSOD- /S1S2- and gSOD- /S1S2+ (Fig. 1). This indicates that excess muscle-derived IGF-1 does not alter the course of motor deterioration in the gSOD+ model.

Motor performance was also tested in CNS IGF-1 over-expressing mice; controls included gSOD- /IGF2/1- and gSOD- /IGF-2/1+ Tg. The gSOD+ /IGF2/1- mice showed a marked decrease in motor performance at day 107 at 18 rpm and at day 128 at 10 rpm compared with both gSOD- /IGF2/1- and gSOD- /IGF2/1+. The gSOD+ / IGF-2/1+ Tg mice showed decreased motor performance at day 107 at 18 rpm and at day 114 at 10rpm compared with the same control groups (Fig. 1). These results indicate that excess brain derived IGF-1 does not alter the course of motor performance deficits in the gSOD+ mouse model.

The lack of any significant beneficial effects of specific overexpression of IGF-1 in CNS and muscle on motor performance in the gSOD+ mouse model may be due to intrinsic variability among gSOD+ mice. To examine this, we used the bSOD+ strain that has a more homogenous phenotype (Jackson Labs). For the bSOD+ mice, we started the rotarod test 20 days earlier than with the gSOD+ model (initiated on day 87 vs. day 107). The rationale for this is that bSOD+ mice have not been studied as extensively and the clinical onset of the disease was unknown. In this model, we determined that deficits in motor performance started at day 87 at both 10 and 18 rpm. The bSOD+ / IGF-2/1+ mice showed a decline in motor performance at day 87 at 18 rpm and on day 141 at 10 rpm (Fig. 2). These results are similar to the bSOD+ / IGF2/1- mice which showed a decrease in performance at day 87 at 18 rpm and on day 141 at 10 rpm. The bSOD- / IGF-2/1+ Tg and bSOD- /IGF2/1- mice were used as background

controls. These results indicate that IGF-1 overexpression in the CNS does not improve motor performance in bSOD+ mice.

Life-span

In this experiment, we examined the effects of CNS and muscle IGF-1 overexpression on the survival of bSOD and gSOD mouse models. The average survival time for gSOD+/S1S2⁻, gSOD+/S1S2, gSOD+/IGF-2/1⁻, and gSOD+/IGF-2/1⁺ was 129, 120, 142, and 135 days, respectively. The same groups of bSOD mice were analyzed and their average survival was 153, 148, 155, and 143 days, respectively. While a significant difference was found between gSOD+/S1S2⁺ and gSOD+/S1S2⁻ and between bSOD+/IGF2/1⁺ and bSOD+/IGF2/1⁻, this difference indicates that hybrid IGF-1 transgenic mice actually had a slightly shorter life span than their hybrid wild type counterparts. In addition, no significant differences in life span were detected between gSOD+/IGF2/1⁺ and gSOD+/IGF2/1⁻ or between bSOD+/S1S2⁺ and bSOD+/S1S2⁻. These results indicate that sustained and exclusive over-expression of IGF-1 in neither muscle nor CNS prolongs life-span in the ALS mouse models used here.

Motor Neuron Survival

To analyze motor neuron death, we examined sections of the mid-lumbar spinal cord in all four models (Fig. 4). The spinal cord showed loss and atrophy of motor neurons in both SOD+/IGF2/1⁺ mice and SOD+/IGF2/1⁻ mice, indicating that the presence of IGF-1 did not deter disease progression. In contrast, SOD-/IGF2/1⁻ and SOD-/IGF2/1⁺ both maintained motor neuron integrity. Similar results were recorded in the facial nucleus of the SOD/IGF2/1 hybrid mice (data not shown).

We counted motor neurons in the lumbar spinal cord of bSOD mice crossed with CNS or muscle IGF-1 transgenics (Fig. 5). In the lumbar spinal cord, the mean motor neuron counts for bSOD+/IGF2/1⁺, bSOD+/IGF2/1⁻, bSOD-/IGF2/1⁺, bSOD-/IGF2/1⁻ mice were 1243, 1272, 2760, and 2558, respectively. Although no significant differences were observed between the first two and the last two groups, the SOD+ mice exhibited significantly lower motor neuron numbers than SOD- mice (Fig. 5A). Similarly, mean motor neuron counts for hybrid bSOD+ crossed with muscle IGF-1 Tg-mice, bSOD+/S1S2⁺, bSOD+/S1S2⁻, bSOD-/S1S2⁺, and bSOD-/S1S2⁻ mice were 1348, 1246, 2900, and 3157, respectively (Fig. 5B). Again, no significant differences in motor neuron counts were observed between SOD+/S1S2⁺ and SOD+/S1S2⁻ mice or between SOD-/S1S2⁺ and SOD-/S1S2⁻ mice, whereas, the number of motor neurons was significantly lower in SOD+ compared to SOD- mice. These results indicate that localized and sustained IGF-1 overexpression either in the CNS or skeletal muscle does not prevent the loss of lumbar spinal cord motor neurons in SOD+ mice.

Soma Size

ALS has been associated with progressive and severe spinal motor neuron atrophy and cell death (Martin et al., 2007). To examine IGF-1's effect on spinal motor neuron atrophy, we measured and compared spinal cord motor neuron soma size across the same multiple genotypes described above. SOD mutant mice (bSOD+/IGF2/1⁻) exhibit a decreased number of large-sized motor neurons when compared with control (bSOD-/IGF2/1⁻) (Fig. 6 A, B). IGF-1 overexpressed in CNS does not modify motor neuron soma size in bSOD-/IGF2/1⁺ mice (Fig. 6 A, C), and does not prevent the loss of large-sized motor neurons (Fig. 6 A, D). Similarly, Figure 7 shows that SOD+ mice (bSOD+/S1S2⁻) have lost large motor neurons compared to SOD- mice (bSOD-/S1S2⁻) (Fig. 7 A, B). IGF-1 expressed in muscle does not change motor neuron soma size in bSOD-/S1S2⁺ mice (Fig. 7 A, C) and it does not prevent motor neuron atrophy or loss in bSOD-/S1S2⁻ vs. bSOD+/S1S2⁺ mice (Fig. 7 A, D).

IGF-1 expression

To determine IGF-1 expression in SOD+ mice crossbred with CNS and muscle IGF-1 transgenic mice, we measured IGF-1 concentrations in various tissues by RIA between days 116 and 203. We choose to measure IGF-1 concentrations between days 116 and 203 to verify that IGF-1 was sustained at high levels after the onset of motor deficits. Fig. 8 compares human IGF-1 concentration in brain, cerebellum, and spinal cord in bSOD+ and bSOD− mice crossbred with CNS IGF-1 Tg mice (IGF2/1). IGF-1 was highly expressed in the brain of bSOD+/IGF2/1+ mice. No significant difference was observed between this group and bSOD−/IGF2/1+ mice. Both transgenic mouse models expressed significantly higher IGF-1 levels than bSOD+/IGF2/1− mice. Similarly, in the cerebellum, IGF-1 was highly expressed in bSOD+/IGF2/1+ mice. No significant difference was observed in IGF-1 expression between this group and bSOD−/IGF2/1+ mice. However, these two groups expressed significantly more IGF-1 than bSOD+/IGF2/1− mice. In the spinal cord, IGF-1 was highly expressed in bSOD+/IGF2/1+ mice. While there was a difference in IGF-1 expression between this group and bSOD−/IGF2/1+ mice, both transgenic hybrid mouse models expressed more IGF-1 than the bSOD−/IGF2/1− mice. Figure 9 compares human IGF-1 concentration measured in the extensor digitorum longus (EDL), soleus muscles, and spinal cords of bSOD+ and bSOD− mice crossbred with muscle-derived IGF-1 Tg mice (S1S2). In the EDL muscle, IGF-1 was highly expressed in bSOD+/S1S2+ and bSOD−/S1S2+ mice. Although there was a difference in expression of IGF-1 between these groups, the bSOD+/S1S2+ transgenic mice also produced significantly more IGF-1 than the bSOD+/S1S2− and bSOD−/S1S2+ mice. In the soleus muscle, IGF-1 was highly expressed in bSOD+/S1S2+ and bSOD−/S1S2+. However, no significant difference in IGF-1 expression was found between these groups versus the bSOD−/S1S2− or bSOD+/S1S2− mice. This may result from the low level of baseline IGF-1 expression in slow twitch muscles such as the soleus (Coleman, personal communication). In addition, IGF-1 concentration in the spinal cord of S1S2 mice was measured and no significant differences were found between bSOD+/S1S2− and bSOD+/S1S2+ mice.

We did not detect any significant differences in IGF-1 expression in the heart, liver, and serum, between transgenic and nontransgenic mice (data not shown). These data are consistent with a specific overexpression of IGF-1 in the CNS and muscle.

Brain, cerebellum, brain stem, and spinal cord weights

The lack of effect of IGF-1 overexpression on pathophysiology in ALS mice may result from a reduced effect of the trophic factor in hybrid mice. Therefore, we also examined whether IGF-1 has any other biological effects on the brain, cerebellum, brain stem, or spinal cord. For example, it has been reported that increased IGF-1 expression is associated with increased organ weight due to increased neuron numbers (Ye et al., 1996) (Hodge et al., 2005) (Ozdinler and Macklis, 2006). As shown in Figure 10, brain, cerebellum, brain stem, and spinal cord weights in SOD−/IGF2/1+ and SOD+/IGF2/1+ were significantly increased when compared to SOD−/IGF2/1− and SOD+/IGF2/1− mice. This increase in organ weight in IGF-1 transgenic mice indicates that IGF-1 is in fact biologically active in the transgenic hybrid mice.

DISCUSSION

Despite the sustained overexpression of IGF-1 in either skeletal muscle or the CNS of mice expressing a mutant form (G93A) of the human SOD-1 gene, these animals were indistinguishable from control SOD-1 mice in several measures of pathophysiology that characterize this mouse model of familial ALS. Deficits in motor performance, decreased life span, motor neuron atrophy and loss of motor neurons were all unaffected by IGF-1 overexpression. This failure to detect any effects of CNS or muscle overexpression of IGF-1

in the SOD-1 mouse model of ALS was surprising in that several previous studies have reported beneficial effects of IGF-1 in this same mouse model.

In one study intramuscular injection of an AAV construct expressing IGF-1 was retrogradely transported to the spinal cord of SOD-1 mice and shown to increase lifespan, improve motor performance and reduce motor neuron atrophy and degeneration (Kaspar et al., 2003). In another study SOD-1 mice that overexpress IGF-1 in skeletal muscle were also reported to have enhanced motor performance, increased life-span and reduced degeneration of motor neurons (Dobrowolny et al., 2005). Finally, continuous intrathecal delivery of exogenous IGF-1 to SOD-1 mice delayed deficits in motor performance, extended life-span and reduced motor neuron loss (Nagano et al., 2005). However, despite the positive effects of IGF-1 in these studies, the benefits were transient and the IGF-1 treated SOD-1 mice in all three studies died prematurely compared to WT mice. Nonetheless, the positive effects observed in these three studies differ significantly from the uniformly lack of effect of IGF-1 in the present study.

Some possible differences between the present study and these three studies that could potentially account for the discrepant results include: genetic background; mode of IGF-1 administration; promoters used for muscle-specific overexpression; differential access of motor neurons to IGF-1 overexpression in muscle or CNS; and lower levels of IGF-1 overexpression in the present study.

Although the muscle IGF-1 mice used here were on a FVB background, the CNS IGF-1 mice were on a C57BL/6 background similar to the SOD-1 mice in the three studies cited above. Our mode of IGF-1 administration also may be a source for differences in that it is possible that continuous exposure to IGF-1 throughout development could alter receptor expression and hence dampen IGF-1's effect. However, muscle IGF-1 transgenic mice used by Dobrowolny et al. (2005) were also exposed to IGF-1 from birth and showed a positive effect from IGF-1. In the study by Dobrowolny et al., (2005), a myosin light chain (mlc) promoter was used to drive skeletal muscle expression of IGF-1, whereas we used an alpha actin skeletal muscle promoter (Dobrowolny et al., 2005). However, both promoters result in muscle-specific overexpression of IGF-1 in primarily fast twitch-muscles and both induce skeletal muscle hypertrophy. Although exogenous IGF-1 injected into skeletal muscle of the chick embryo (Rind and von Bartheld, 2002) and adult rat (Caroni, 1993) is retrogradely transported, the retrograde transport of IGF-1 wasn't specifically examined in our study or that of Dobrowolny and colleagues. In the present study, IGF-1 levels in the spinal cord as measured by RIA were similar in bSOD+/S1S2- and bSOD+/S1S2+ mice. Even in the absence of retrograde transport, however, target-derived neurotrophic factors can induce retrograde signaling cascades (Zweifel et al., 2005). For example, IGF-1 receptors are expressed on motor axons and terminals (Caroni, 1993) and in a previous study we demonstrated that muscle-specific overexpression of IGF-1 can affect motor terminals and innervation (Messi and Delbono, 2003). Additionally, the application of exogenous IGF-1 at the site of peripheral axotomy in the sciatic and facial nerve of neonatal mice and rats also rescues motor neurons from injury induced cell death (Hughes et al., 1993; Li et al., 1994). Finally, intramuscular injection of IGF-1 binding proteins that sequester and reduce the biological activity of endogenous IGF-1 in muscle, perturbs neuromuscular innervation, consistent with a role for endogenous muscle derived IGF-1 on motor neurons (Caroni, 1993; D'Costa et al., 1998; Rind and von Bartheld, 2002). Accordingly, it seems unlikely that our failure to observe any effect of IGF-1 overexpression in muscle on disease progression in SOD-1 mice is due to a total lack of motor neuron access to IGF-1. However, we cannot exclude the possibility that our use of the alpha actin promoter precluded the retrograde transport of IGF-1 and that retrograde transport may be critical for preventing motor neuron deficits in SOD-1 mice. For example, in a related study comparing muscles vs CNS overexpression of GDNF, it was indicated that retrograde transport of GDNF may be critical for neuroprotection in SOD-1 mice (Li et al., 2007); GDNF was

neuroprotective following muscle but not CNS overexpression. Although this might also explain why CNS overexpression of IGF-1 was ineffective in the present study, it does not explain why CNS (intrathecal) infusion of IGF-1 was neuroprotective in SOD-1 mice (Nagano et al., 2005) or why in our study, CNS overexpression of IGF-1 resulted in increased growth of brain and spinal cord, a clear index of biological activity in the CNS.

Although we are unable to provide a definitive explanation for our failure (in contrast to previous studies) to detect a neuroprotective effect of IGF-1 in a mouse model of ALS, these negative results, together with a negative results of one of two clinical trials using IGF-1 with ALS patients (Borasio et al., 1998), indicate a note of caution in the future use of IGF-1 to treat motor neuron disease. However, since SOD+ mouse models do not always represent human disease, it is still possible that IGF-1 may have some beneficial effects in humans, the current clinical trial will be crucial to understanding how the route of IGF-1 effects the disease course in humans.

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Reference List

- Borasio GD, Robberecht W, Leigh PN, Emile J, Guiloff RJ, Jerusalem F, Silani V, Vos PE, Wokke JH, Dobbins T. A placebo-controlled trial of insulin-like growth factor-I in amyotrophic lateral sclerosis. European ALS/IGF-I Study Group. *Neurology* 1998;51:583–586. [PubMed: 9710040]
- Buss RR, Gould TW, Ma J, Vinsant S, Prevette D, Winseck A, Toops KA, Hammarback JA, Smith TL, Oppenheim RW. Neuromuscular development in the absence of programmed cell death: phenotypic alteration of motoneurons and muscle. *J Neurosci* 2006;26:13413–13427. [PubMed: 17192424]
- Caroni P. Activity-sensitive signaling by muscle-derived insulin-like growth factors in the developing and regenerating neuromuscular system. *Ann N Y Acad Sci* 1993;692:209–222. [PubMed: 8215024]
- Clarke PG, Oppenheim RW. Neuron death in vertebrate development: in vitro methods. *Methods Cell Biol* 1995;46:277–321. [PubMed: 7609654]
- Coleman ME, DeMayo F, Yin KC, Lee HM, Geske R, Montgomery C, Schwartz RJ. Myogenic vector expression of insulin-like growth factor I stimulates muscle cell differentiation and myofiber hypertrophy in transgenic mice. *J Biol Chem* 1995;270:12109–12116. [PubMed: 7744859]
- D'Costa AP, Prevette DM, Houenou LJ, Wang S, Zackenfels K, Rohrer H, Zapf J, Caroni P, Oppenheim RW. Mechanisms of insulin-like growth factor regulation of programmed cell death of developing avian motoneurons. *J Neurobiol* 1998;36:379–394. [PubMed: 9733073]
- Dai Z, Takahashi SI, Van Wyk JJ, D'Ercole AJ. Creation of an autocrine model of insulin-like growth factor-I action in transfected FRTL-5 cells. *Endocrinology* 1992;130:3175–3183. [PubMed: 1375893]
- Dobrowolny G, Giacinti C, Pelosi L, Nicoletti C, Winn N, Barberi L, Molinaro M, Rosenthal N, Musaro A. Muscle expression of a local Igf-1 isoform protects motor neurons in an ALS mouse model. *J Cell Biol* 2005;168:193–199. [PubMed: 15657392]
- Gould TW, Buss RR, Vinsant S, Prevette D, Sun W, Knudson CM, Milligan CE, Oppenheim RW. Complete dissociation of motor neuron death from motor dysfunction by Bax deletion in a mouse model of ALS. *J Neurosci* 2006;26:8774qq–8786. [PubMed: 16928866]
- Gurney ME. Transgenic-mouse model of amyotrophic lateral sclerosis. *N Engl J Med* 1994;331:1721–1722. [PubMed: 7832899]
- Hodge RD, D'Ercole AJ, O'Kusky JR. Increased expression of insulin-like growth factor-I (IGF-I) during embryonic development produces neocortical overgrowth with differentially greater effects on specific cytoarchitectonic areas and cortical layers. *Brain Res Dev Brain Res* 2005;154:227–237.

- Hughes RA, Sendtner M, Thoenen H. Members of several gene families influence survival of rat motoneurons in vitro and in vivo. *J Neurosci Res* 1993;36:663–671. [PubMed: 8145295]
- Julien JP, Kriz J. Transgenic mouse models of amyotrophic lateral sclerosis. *Biochim Biophys Acta* 2006;1762:1013–1024. [PubMed: 16675207]
- Kaspar BK, Llado J, Sherkat N, Rothstein JD, Gage FH. Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. *Science* 2003;301:839–842. [PubMed: 12907804]
- Lai EC, Felice KJ, Festoff BW, Gawel MJ, Gelinas DF, Kratz R, Murphy MF, Natter HM, Norris FH, Rudnicki SA. Effect of recombinant human insulin-like growth factor-I on progression of ALS. A placebo-controlled study. The North America ALS/IGF-I Study Group. *Neurology* 1997;49:1621–1630. [PubMed: 9409357]
- Lewis ME, Neff NT, Contreras PC, Stong DB, Oppenheim RW, Grebow PE, Vaught JL. Insulin-like growth factor-I: potential for treatment of motor neuronal disorders. *Exp Neurol* 1993;124:73–88. [PubMed: 8282084]
- Li L, Oppenheim RW, Lei M, Houenou LJ. Neurotrophic agents prevent motoneuron death following sciatic nerve section in the neonatal mouse. *J Neurobiol* 1994;25:759–766. [PubMed: 8089654]
- Li W, Brakefield D, Pan Y, Hunter D, Myckatyn TM, Parsadanian A. Muscle-derived but not centrally derived transgene GDNF is neuroprotective in G93ASOD1 mouse model of ALS. *Exp Neurol* 2007;203:457–471. [PubMed: 17034790]
- Martin LJ, Liu Z, Chen K, Price AC, Pan Y, Swaby JA, Golden WC. Motor neuron degeneration in amyotrophic lateral sclerosis mutant superoxide dismutase-1 transgenic mice: mechanisms of mitochondriopathy and cell death. *J Comp Neurol* 2007;500:20–46. [PubMed: 17099894]
- Matsumoto A, Okada Y, Nakamichi M, Nakamura M, Toyama Y, Sobue G, Nagai M, Aoki M, Itoyama Y, Okano H. Disease progression of human SOD1 (G93A) transgenic ALS model rats. *J Neurosci Res* 2006;83:119–133. [PubMed: 16342121]
- Messi ML, Delbono O. Target-derived trophic effect on skeletal muscle innervation in senescent mice. *J Neurosci* 2003;23:1351–1359. [PubMed: 12598623]
- Mohajeri MH, Figlewicz DA, Bohn MC. Selective loss of alpha motoneurons innervating the medial gastrocnemius muscle in a mouse model of amyotrophic lateral sclerosis. *Exp Neurol* 1998;150:329–336. [PubMed: 9527903]
- Moreno RJ, Messi ML, Zheng Z, Wang ZM, Ye P, D'Ercole JA, Delbono O. Role of sustained overexpression of central nervous system igf-I in the age-dependent decline of mouse excitation-contraction coupling. *J Membr Biol* 2006;212:147–161. [PubMed: 17334835]
- Mulder DW, Kurland LT, Offord KP, Beard CM. Familial adult motor neuron disease: amyotrophic lateral sclerosis. *Neurology* 1986;36:511–517. [PubMed: 3960325]
- Nagano I, Ilieva H, Shiote M, Murakami T, Yokoyama M, Shoji M, Abe K. Therapeutic benefit of intrathecal injection of insulin-like growth factor-I in a mouse model of Amyotrophic Lateral Sclerosis. *J Neurol Sci* 2005;235:61–68. [PubMed: 15990113]
- Ozdinler PH, Macklis JD. IGF-I specifically enhances axon outgrowth of corticospinal motor neurons. *Nat Neurosci* 2006;9:1371–1381. [PubMed: 17057708]
- Renganathan M, Messi ML, Schwartz R, Delbono O. Overexpression of hIGF-1 exclusively in skeletal muscle increases the number of dihydropyridine receptors in adult transgenic mice. *FEBS Lett* 1997;417:13–16. [PubMed: 9395065]
- Renganathan M, Messi ML, Delbono O. Overexpression of IGF-1 exclusively in skeletal muscle prevents age-related decline in the number of dihydropyridine receptors. *J Biol Chem* 1998;273:28845–28851. [PubMed: 9786885]
- Rind HB, von Bartheld CS. Target-derived cardiotrophin-1 and insulin-like growth factor-I promote neurite growth and survival of developing oculomotor neurons. *Mol Cell Neurosci* 2002;19:58–71. [PubMed: 11817898]
- Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng HX, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 1993;362:59–62. [PubMed: 8446170]

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- Ye P, Xing Y, Dai Z, D'Ercole AJ. In vivo actions of insulin-like growth factor-I (IGF-I) on cerebellum development in transgenic mice: evidence that IGF-I increases proliferation of granule cell progenitors. *Brain Res Dev Brain Res* 1996;95:44–54.
- Zweifel LS, Kuruvilla R, Ginty DD. Functions and mechanisms of retrograde neurotrophin signalling. *Nat Rev Neurosci* 2005;6:615–625. [PubMed: 16062170]

Figure 1. Motor performance analysis (rotarod test) in SOD+ and SOD- mice crossed with either wild type or IGF-1 transgenic mice. A-D. Probability to fall in gSOD+ and gSOD- mice crossed with either wild type or muscle specific IGF-1 transgenic mice. EH. Probability to fall in gSOD+ and gSOD- mice crossed with either wild type or CNS IGF-1 transgenic mice.

Figure 2.

Probability to fall in bSOD+ and bSOD- crossed with either wild type or CNS IGF-1 transgenic mice at 18 and 10 rpm. A. bSOD-/IGF2/1- mice B. bSOD-/IGF2/1+ C. bSOD+/IGF2/1- mice D. bSOD+/IGF2/1+ mice.

Figure 3.

Kaplan-Meier survival analysis of SOD+ and SOD- mice crossbred with muscle or CNS IGF-1 transgenic mice. The dashed line represents SOD+ mice crossed with wild type. The solid line represents SOD+ mice crossed with IGF-1 (either expressed in muscle or CNS) transgenic mice. A. Survival of gSOD+ and gSOD- mice crossed with either wild type or muscle IGF-1 transgenic mice. Wild type mice lived slightly longer than muscle IGF-1 transgenic mice ($p < 0.05$). B. Survival of gSOD+ and gSOD- mice crossed with either wild type or CNS IGF-1 transgenic mice. No significant difference was found ($p > 0.05$). C. Survival of bSOD+ and bSOD- mice crossed with either wild type or CNS IGF-1 transgenic mice. Wild type mice lived slightly longer than CNS IGF-1 transgenic mice ($p < 0.05$). D. Survival of bSOD+ and bSOD- mice crossed with either wild type or muscle IGF-1 transgenic mice. No significant difference was found ($p > 0.05$).

Figure 4.

Sections from the mid-lumbar (L) spinal cord (L3-4) of WT (SOD⁻/IGF⁻) and transgenic mice on day 154. Note the decreased size of the ventral horn (VH), the loss of motor neurons and the atrophy of surviving motor neurons in the SOD⁺ mice. Inset: large (asterisks) and small (arrows) motor neurons and non-neuronal cells (arrowheads). LWM, lateral white matter; VWM, ventral white matter; dotted line, border between the VH and intermediate gray matter. Scale bar=100µm for all images and 10µm for the inset. Scale bar=100µm for all images.

Figure 5.

Motor neuron survival in SOD+ and SOD- mice crossbred with muscle or CNS IGF-1 transgenic mice: A. Lumbar spinal cord motor neuron counts for bSOD/IGF2/1 hybrid mice B. Lumbar spinal cord motor neuron counts for bSOD/S1S2 hybrid mice. Number of mice are indicated. Differences between bSOD+/IGF2/1- and bSOD-/IGF2/1- mice were statistically significant ($P < 0.001$) but not between bSOD+/IGF2/1- and bSOD+/IGF2/1+ mice ($P > 0.05$). Similarly, differences between bSOD+/S1S2- and bSOD-/S1S2- mice were statistically significant ($P < 0.001$) but not between bSOD+/S1S2- and bSOD+/S1S2+ mice ($P > 0.05$).

Figure 6. Motor neuron soma size (μm^2) analysis in SOD+ and SOD- mice crossed with either CNS IGF-1 transgenic mice or wild type. A. bSOD-/IGF2/1- mice B. bSOD+/IGF2/1- mice C. bSOD-/IGF2/1+ mice D. bSOD+/IGF2/1+ mice.

Figure 7. Motor neuron soma size (μm^2) analysis in bSOD+ and bSOD- mice crossed with either muscle IGF-1 transgenic mice or wild type. A. bSOD-/S1S2- mice B. bSOD+/S1S2- mice C. bSOD-/S1S2+ mice D. bSOD+/S1S2+ mice

Figure 8. IGF-1 concentration measured in brain, cerebellum, and spinal cord for bSOD/IGF2/1 mice. The number of mice used is indicated. A statistical significant difference in IGF-1 concentration was found between bSOD+/IGF2/1- and bSOD+/IGF2/1+ mice in brain, cerebellum, and spinal cord ($P < 0.001$, 0.001 , and 0.05 , respectively).

Figure 9.

IGF-1 concentration measured in extensor digitorum longus (EDL), soleus, and spinal cord for bSOD/S1S2 mice. The number of mice used is indicated. In EDL, there was a significant difference in IGF-1 concentration found between bSOD+/S1S2- and bSOD+/S1S2+ mice ($P < 0.05$). However, no significant differences were found between bSOD+/S1S2- and bSOD+/S1S2+ mice in IGF-1 concentration ($P > 0.05$) in soleus muscle. Similarly, no significant differences were found in the spinal cord between bSOD+/S1S2- and bSOD+/S1S2+ mice.

Figure 10.

Brain, cerebellum, brain stem, and spinal cord weight in SOD/IGF2/1. The number of mice studied were 8, 9, 5, 9 for IGF2/1+/GSOD+, IGF-2/1-/GSOD-, IGF-2/1-/GSOD+, and IGF-2/1+/SOD- mice, respectively. Significant differences between SOD+/IGF2/1+ and SOD+/IGF2/1- mouse organ weights were found in brain, cerebellum, brain stem, and spinal cord ($P < 0.001$, 0.001 , 0.001 , and 0.05 respectively). Similarly, significant differences between SOD-/IGF2/1- and SOD-/IGF2/1+ mouse organ weights were found in brain, cerebellum, brain stem, and spinal cord ($P < 0.001$, 0.001 , 0.05 , and 0.05 , respectively).