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Lead exposure stimulates VEGF expression in the spinal cord and extends survival in a mouse model of ALS

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ABSTRACT

Exposure to environmental lead (Pb) is a mild risk factor for amyotrophic lateral sclerosis (ALS), a paralytic disease characterized by progressive degeneration of motor neurons. However, recent evidence has paradoxically linked higher Pb levels in ALS patients with longer survival. We investigated the effects of low-level Pb exposure on survival of mice expressing the ALS-linked superoxide dismutase-1 G93A mutation (SOD1^{G93A}). SOD1^{G93A} mice exposed to Pb showed longer survival and increased expression of VEGF in the ventral horn associated with reduced astrocytosis. Pretreatment of cultured SOD1^{G93A} astrocytes with low, non toxic Pb concentrations upregulated VEGF expression and significantly abrogated motor neuron loss in coculture, an effect prevented by neutralizing antibodies to VEGF. The actions of Pb on astrocytes might explain its paradoxical slowing of disease progression in SOD1^{G93A} mice and the improved survival of ALS patients. Understanding how Pb stimulates astrocytic VEGF production and reduces neuroinflammation may yield a new therapeutic approach for treating ALS.

Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder characterized by the selective death of motor neurons, resulting in progressive paralysis and death. The cause of the disease remains unknown for the majority of sporadic cases of ALS. The discovery of missense mutations in the gene encoding the antioxidant enzyme Cu/Zn superoxide dismutase-1 (SOD1) in a subset of patients with familial ALS (Rosen et al., 1993) stimulated research in transgenic animal models expressing different SOD1 mutations though the pathogenic mechanism underlying the toxicity of SOD1 mutants remains controversial.

Studies in transgenic mice have shown evidence for a non-cell autonomous toxicity of SOD1 mutations. Decreasing mutant SOD1 expression in astrocytes or microglia (Boillee et al., 2006; Yamanaka et al., 2008), and grafting wild-type macrophages to mutant SOD1 mice (Beers et al., 2006), increase the lifespan of mutant SOD1 mice. In addition, *in vitro* studies have shown that astrocytes expressing the SOD1^{G93A} mutation exert toxic influence to non-transgenic motor neurons (Vargas et al., 2006; Nagai et al., 2007; Di Giorgio et al., 2007; Cassina et al., 2008), suggesting that these glial cells can play a disease-modifying role in ALS pathogenesis (Barbeito et al., 2004).

Occupational or environmental exposure to a variety of toxicants including heavy metals has been evaluated as potential causes of ALS. Exposure to the heavy metal lead (Pb) and in particular blood and bone content of Pb was associated with an increased risk of ALS (Kamel et al., 2005). More recently, epidemiological data also showed that Pb blood and bone levels positively correlate with longer survival in ALS patients after diagnosis (Kamel et al., 2008), suggesting Pb exposure may paradoxically delay the disease progression.

Pb is a widely spread environmental heavy metal with no known specific biological function. Pb has been shown to induce acute and chronic neurotoxicity, particularly during CNS development (Cory-Slechta and Pound, 1995; Bellinger, 2008). Both neuronal and glial cells seem to be affected in Pb neurotoxicity (Tiffany-Castiglioni and Qian, 2001). However, the toxicity of Pb on spinal cord motor neurons and astrocytes is presently unknown as is its potential role in the etiology of ALS.

Experimental evidence supports the view that astrocytes can sequester and buffer Pb in the CNS, preventing further diffusion of the metal to the neuronal compartment and subsequent neurotoxicity or altered synaptic transmission (Tiffany-Castiglioni, 1993; Tiffany-Castiglioni and Qian, 2001). In particular, astrocytes are the cells that preferentially induce cytoprotective and antioxidant gene expression in response to Pb (Cabell et al., 2004). Thus, available

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evidence supports the view that astrocytes are key targets of Pb and respond to it by inducing neuroprotective pathways.

We began investigating the hypothesis that Pb would accelerate ALS in the SOD1^{G93A} mice, but were surprised to find increased survival and reduced astrocytic reactivity. We further investigated this paradoxical finding in astrocyte/motor neuron cocultures and found an up-regulation of vascular endothelial growth factor (VEGF) after Pb treatment. Our results suggest that Pb activates a novel pathway able to reduce neuroinflammation and slow neurodegeneration in ALS.

Materials and methods

Materials

Culture media and serum were obtained from Invitrogen (Carlsbad, CA). All other reagents were from Sigma unless otherwise specified.

Animals

Procedures using laboratory animals were in accordance with international guidelines and were approved by the Institutional Animal Committee. Sprague–Dawley SOD1G93A L26H rats were kindly provided by Dr David S. Howland (Wyeth Research, Princeton, NJ, USA) (Howland et al., 2002). Transgenic ALS mice carrying the G93A mutation for human SOD1, strain B6SJL-TgN (SOD1-G93A)1Gur (Gurney et al., 1994), were obtained from Jackson Laboratory (Bar Harbor, ME, USA) and genotyped as previously described (Vargas et al., 2005). Mice were housed under controlled conditions with free access to food and water. Male transgenic ($n=8$ per group) and non-transgenic ($n=7$ per group) littermates were divided randomly into the following groups: A) control group which received sodium acetate in drinking water, at a concentration of 200 ppm, B) Pb treatment group, administered with Pb acetate (PbAc) in drinking water at a concentration of 200 ppm. The treatment was performed from weaning (21–25-days-old) to death. Animals were observed weekly for onset of disease symptoms, as well as progression to death. Onset of disease was scored as the first observation of abnormal gait or overt hind limb weakness. End-stage of the disease was scored as complete paralysis of both hind limbs and the inability of the animals to right themselves after being turned on a side.

Blood lead levels analysis

Blood was collected at disease onset (between days 90 and 95). Pb levels were analyzed by Electrothermal Atomic Absorption Spectrometry (ETAAS) with a VARIAN SpectrAA-55. Analytical conditions were validated with quality assurance/quality control (QA/QC) procedure standards guidelines (Parsons and Chisolm, 1997; CDC, 2005).

Cell cultures

Primary rat spinal cord astrocyte cultures were prepared from transgenic SOD1^{G93A} and non-transgenic 1-day-old pups, genotyped by PCR, as previously described (Cassina et al., 2002; Vargas et al., 2006). Briefly, cells were plated at a density of 2×10^4 cells/cm^2 in 35-mm Petri dishes or 24-well plates (Nunc, Naperville, IL, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, HEPES (3.6 g/l), penicillin (100 IU/ml) and streptomycin (100 μg/ml). Astrocyte monolayers were >98% pure as determined by GFAP immunoreactivity.

Motor neuron cultures were prepared from embryonic day 15 (E15) rat spinal cord by a combination of metrizamide gradient centrifugation and immunopanning with the monoclonal antibody IgG192 against p75 neurotrophin receptor as described (Cassina et al., 2002), then plated on rat astrocyte monolayers at a density of 300 cells/cm^2 and maintained for 48 h in L15 supplemented medium as described (Cassina et al., 2002).

Treatment of cultures and motor neuron counting

Confluent astrocyte monolayers were changed to L15 supplemented media prior treatment. Stock solution of PbAc (Baker, US) was prepared in distilled water and directly applied to astrocyte monolayers at the indicated concentrations. For coculture experiments, astrocyte monolayers were treated with PbAc for 24 h and motor neurons were plated in fresh L15 supplemented media, after washing twice with Dulbecco's PBS. For neutralization of VEGF biological activity, anti-rat VEGF antibody (1 μg/ml, AF564, R&D Systems) was added to the culture media 2 h after motor neuron plating. Motor neuron survival was assessed after 48 h by directly counting all p75^{NTR} immunoreactive cells displaying neurites longer than 4 cell bodies diameter (Cassina et al., 2002).

Immunoblot analysis

Protein samples extracted from astrocyte monolayers or spinal cord homogenates, were resolved on 12% SDS–polyacrylamide gel and transferred to nitrocellulose membrane (Amersham, Piscataway, NJ, USA) as described (Vargas et al., 2006). VEGF primary antibody (Santa Cruz Biotechnology) was used at 1:250 dilution. Equal protein load was verified by β-actin immunodetection (Sigma, St. Louis, MO) after stripping of the membrane. For spinal cord western blots, five transgenic mice at the age of symptoms onset and the same number of age-matched non-Tg mice were used. Spinal cords were removed and immediately frozen in liquid nitrogen, and processed in SDS 1% plus Complete Protease Inhibitor Cocktail (Roche) for protein extraction.

Immunohistochemistry and immunofluorescence

Mice were transcardially perfused with 4% paraformaldehyde fixative in PBS under deep anesthesia. The spinal cords were post fixed and embedded in Paraplast. Serial sections (5 μm-thick) were submitted to antigen retrieval (microwave in citrate buffer) followed by blockage of endogenous peroxidase activity prior to immunohistochemistry. After permeabilization (0.25% Triton X-100 in 0.1M PBS) and blocking unspecific binding (10% goat serum, 2% BSA, 0.25% Triton X-100 in PBS), slides were incubated with primary antibodies diluted in blocking solution for 48 h at 4 °C. The primary antibodies were mouse monoclonal anti-VEGF (1:100, Santa Cruz Biotechnology) and rabbit polyclonal anti-GFAP (1:600, Sigma). Sections were washed and exposed to secondary antibodies (1–2 h at room temperature), Alexa Fluor^{488} conjugated goat anti-rabbit (Molecular Probes; 1,5 μg/ml) or anti-mouse biotinylated antibody (Jackson Immunoresearch, 1:400), followed by streptavidin–horseradish peroxidase (Jackson Immunoresearch, 1 μg/ml in Tris–HCl, pH 7.4) and developed with 0.5 mg/ml diaminobenzidine plus 0.01% (v/v) hydrogen peroxide in 0.05 M Tris–HCl. Controls were performed by omitting the primary antibody. Images were obtained using an Olympus FV300 confocal microscopy. Quantification of astrogliosis in the lumbar spinal cord was performed on images obtained from every fifth section ($n=15$ from each group) using ImageJ software (NIH). Ventral horn area occupied by GFAP immunofluorescence was measured and expressed as a percentage of total ventral horn area in each section.

Statistics

Survival curves were compared by Kaplan–Meier analysis with the log-rank test using the GraphPad PRISM software, version 4.03.

All culture assays were performed in duplicate and each experiment was repeated at least three times. Quantitative data were expressed as mean ± SEM and ANOVA and Student's *t* test were used for statistical analysis, with $p<0.05$ considered significant. When the normality test failed, comparison of the means was performed by one-way ANOVA on ranks followed by the Kruskal–Wallis test. Data from GFAP were analyzed using a one-way ANOVA for repeated measurements, and compared by all pairwise multiple comparison procedures (Holm–Sidak method). All statistics computations were performed using the Sigma Stat System (1994, Jandel Scientific, San Rafael, CA, USA), or GraphPad InStat software, version 3.06.

Results

Lead exposure increased survival of transgenic SOD1^{G93A} mice. SOD1^{G93A} and non-transgenic mice were treated from weaning until death with a low concentration of PbAc or sodium acetate (vehicle) in drinking water (Fig. 1). Chronic exposure to Pb did not significantly modify disease onset (vehicle: 101 ± 14 days; PbAc: 110 ± 11 days) but significantly increased life expectancy by 12 days (vehicle $n=7$: 125 ± 1; PbAc $n=8$: 137 ± 2 days) (Figs. 1A and B). The Pb treatment caused a 13-fold increase in blood Pb levels as analyzed by electrothermal atomic absorption spectrometry at disease onset. Metal blood concentration in Pb-treated group (27 ± 7.4 μg/dl vs. 2.0 ± 1.0 μg/dl in control mice) is below the accepted level for occupational Pb exposure (40 μg/dl) under Occupational Safety and Health Administration (OSHA) standards (Roscoe et al., 2002). Accordingly, exposed mice behaved normally and did not show apparent signs of Pb intoxication, such as weight loss, disease or premature death, when compared to vehicle-treated mice.

Pb exposure decreased astrocyte GFAP levels in the ventral spinal cord. A group of mice were sacrificed at the first signs of disease onset, as described in Materials and methods, to perform microscopic analysis of the spinal cord. There were no apparent differences in motor neuron number or morphology with respect to mice treated with vehicle only. However, Pb-treated SOD1^{G93A} mice displayed a marked reduction in astrocyte reactivity, determined by GFAP immunolabeling, when compared to vehicle-treated group (Fig. 2A). Quantification of GFAP immunofluorescence showed about 50% reduction in GFAP staining in Pb-treated mouse spinal cord, compared to control littermates (Fig. 2B).

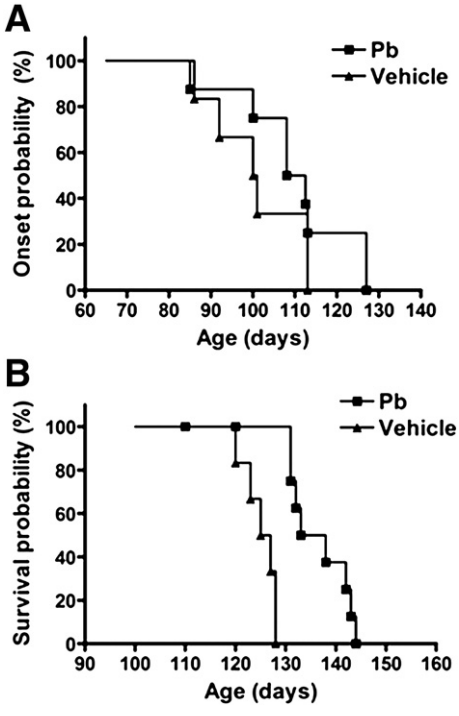

Fig. 1. Exposure to low-Pb levels increases survival of SOD1^{G93A} transgenic mice. PbAc (Pb) or sodium acetate (vehicle) was administered in drinking water from weaning to death as detailed in Materials and methods. (A) Disease onset was not modified by Pb administration when compared to vehicle-treated group (vehicle: 101 ± 14 days; Pb: 110 ± 11 days; Kaplan–Meier curve, $p=0.29$, log-rank test). (B) Administration of Pb resulted in a significant increase in the survival of SOD1^{G93A} transgenic mice when compared to vehicle-treated group (vehicle: 125 ± 1 days; Pb: 137 ± 2 days; Kaplan-Meier curve, $p<0.0001$, log-rank test).

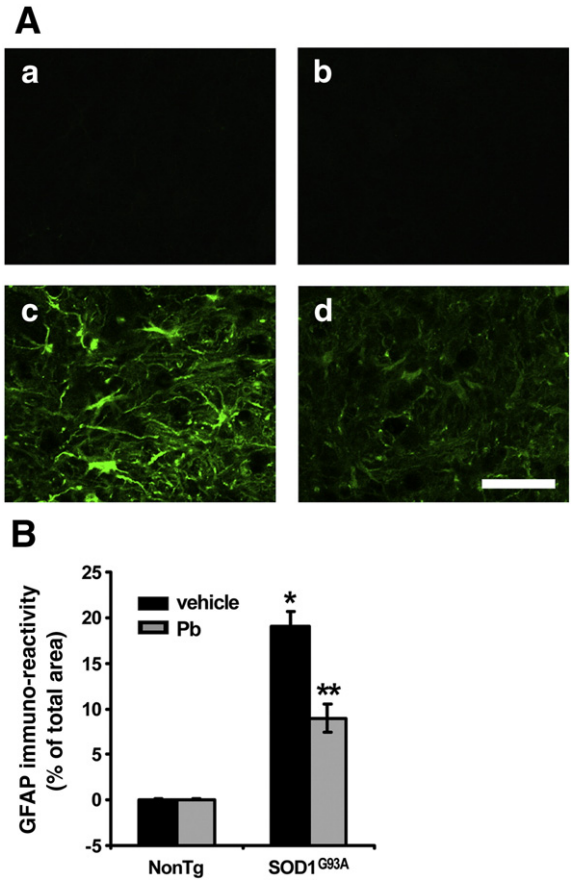

Fig. 2. Effects of Pb exposure on GFAP immunoreactivity in the lumbar spinal cord. (A) GFAP immunofluorescence was almost undetectable in the ventral horns of lumbar spinal cord from non-transgenic animals treated with vehicle (top panels, a), or with Pb treatment (top panels, b). Lower panels show that the increased GFAP immunoreactivity displayed by vehicle-treated SOD1^{G93A} mice (c) was greatly reduced by Pb treatment (d). Scale bar: 40 μm. (B) Quantification of GFAP immunofluorescence in the ventral horn showed a reduction of more than 50% in Pb-treated SOD1^{G93A} animals compared to vehicle-treated transgenic mice. GFAP immunofluorescence was less than 0.1% in PbAc or NaAc treated non-transgenic animals. Values represent the mean ± SEM of measured areas of at least 15 spinal cord sections per group. *$p<0.05$ compared with non-transgenic vehicle-treated group; **$p<0.05$ compared with vehicle-treated SOD1^{G93A} group.

Pb exposure was previously found to induce VEGF gene expression in immortalized human fetal astrocytes (Hossain et al., 2000). Thus, we analyzed VEGF expression in spinal cord of Pb- and vehicle-treated mice. Western blot analysis of spinal cord VEGF showed basal increase of VEGF levels in SOD1^{G93A} mice compared to non-transgenic mice, which was further elevated by 36% following Pb treatment (Fig. 3A). Spinal cord VEGF levels in non-transgenic mice were below the detection level in the current analytical conditions and were not increased by Pb.

In agreement with western blot analysis, VEGF immunohistochemistry showed an increase of VEGF immunoreactivity in the ventral spinal cord of SOD1^{G93A} mice treated with Pb (Fig. 3B). In particular, VEGF immunoreactivity increased in the neuropil around motor neurons. In comparison, VEGF immunoreactivity in non-

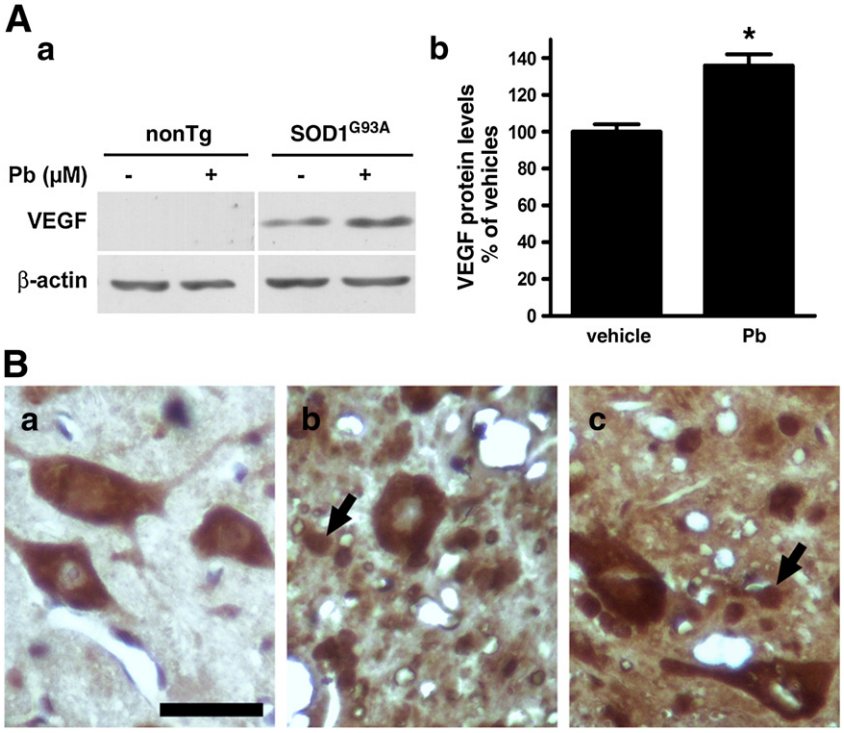

Fig. 3. VEGF expression is increased in the lumbar spinal cord of SOD1^{G93A} transgenic mice exposed to Pb. VEGF expression was analyzed in the lumbar spinal cord of SOD1^{G93A} and non-transgenic mice exposed to PbAc (Pb) or sodium acetate (Vehicle). (A) Representative immunoblots (a) and relative densitometric analysis (b) of VEGF expression in vehicle- and Pb-treated non-transgenic and SOD1^{G93A} mice. Values represent the mean ± SEM of three different experiments compared by one-way ANOVA. *p<0.05 compared with SOD1^{G93A} vehicle-treated group. (B) Representative VEGF immunohistochemistry in the ventral horn of spinal cord. Immunoreactivity is typically detected in motor neuron soma, both in non-transgenic (a) and SOD1^{G93A} (b) mice; note the increase of VEGF immunostaining in the neuropil of SOD1^{G93A} transgenic mice (b), which was augmented by Pb treatment (c). Arrows show VEGF imunoreactive cells surrounding motor neurons. Scale bar: 30 μm.

transgenic mice was limited to the motor neuron cell bodies and was not overtly increased by Pb (Fig. 3B).

Because Pb induced VEGF expression in the neuropil of SOD1^{G93A} spinal cord mice, we further investigated whether Pb induced VEGF in astrocyte cultures. VEGF expression was basally increased in SOD1^{G93A} astrocyte cultures when compared to non-transgenic astrocytes. In addition, a 24 h Pb exposure (10 μM) increased VEGF levels by 2-fold in SOD1^{G93A} astrocytes (Fig. 4). VEGF expression was not detected in non-transgenic astrocyte cultures, which remained below the detection limit even following Pb treatment (Fig. 4).

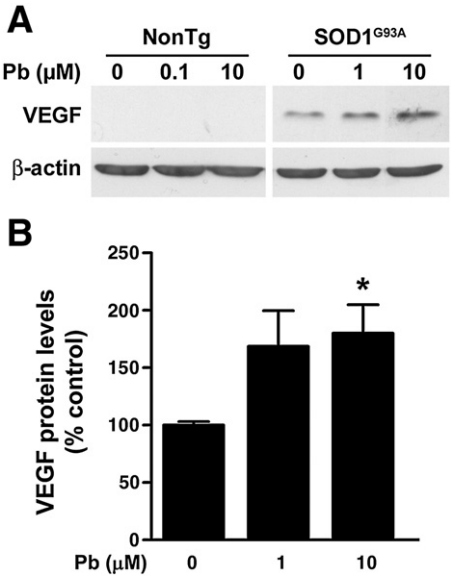

Fig. 4. Lead exposure increases VEGF protein levels in SOD1^{G93A} transgenic astrocytes. (A) Representative western blot of SOD1^{G93A} and non-transgenic astrocytes treated for 24 h with increasing concentrations of PbAc (Pb). Note the increase in VEGF levels with Pb treatment. No signal was detected in non-transgenic astrocytes. (B) Relative densitometric analysis of VEGF expression in SOD1^{G93A} astrocytes. Values represent the mean ± SEM of four different experiments. *p<0.05 compared with SOD1^{G93A} vehicle-treated group.

We have employed our previously validated coculture assay (Pehar et al., 2004) to analyze whether Pb modulated the ability of SOD1^{G93A} astrocytes to promote motor neuron death. Pb exposure to astrocyte monolayers for up to 2 days did not induce cell death or apparent morphological changes in astrocytes as assayed by microscopic analysis, GFAP immunofluorescence and MTT (data not shown). In contrast, isolated embryonic motor neurons maintained with GDNF were vulnerable to Pb applied to the culture media at concentrations ≥10 μM (Fig. 5A). Pre-exposure of SOD1^{G93A} bearing astrocytes to 10 μM Pb for 24 h caused astrocytes to increase motor neuron survival from 64% (vehicle) to 96% (PbAc), thus partially reverting SOD1^{G93A} astrocyte-mediated neurotoxicity (Fig. 5B). This protective effect was prevented by VEGF-blocking antibodies added to the cocultures (Fig. 5B) while vehicle-treated cocultures were unaffected by VEGF-blocking antibodies (Fig. 5B) suggesting that VEGF is necessary for protection. In contrast, pre-exposure of non-transgenic astrocytes to Pb made them unable to support motor neuron survival (Fig. 5C) further indicating the specific effect of Pb on SOD1^{G93A} bearing astrocytes.

Discussion

Paradoxically, low-level Pb exposure was protective in a severe model of ALS, which correlated with higher levels of VEGF in spinal cord. The present finding was unexpected, since Pb is a well-known environmental neurotoxicant and a risk factor for ALS (Kamel et al., 2005). We provide evidence that Pb decreases the toxicity of dysfunctional astrocytes occurring in familial ALS by

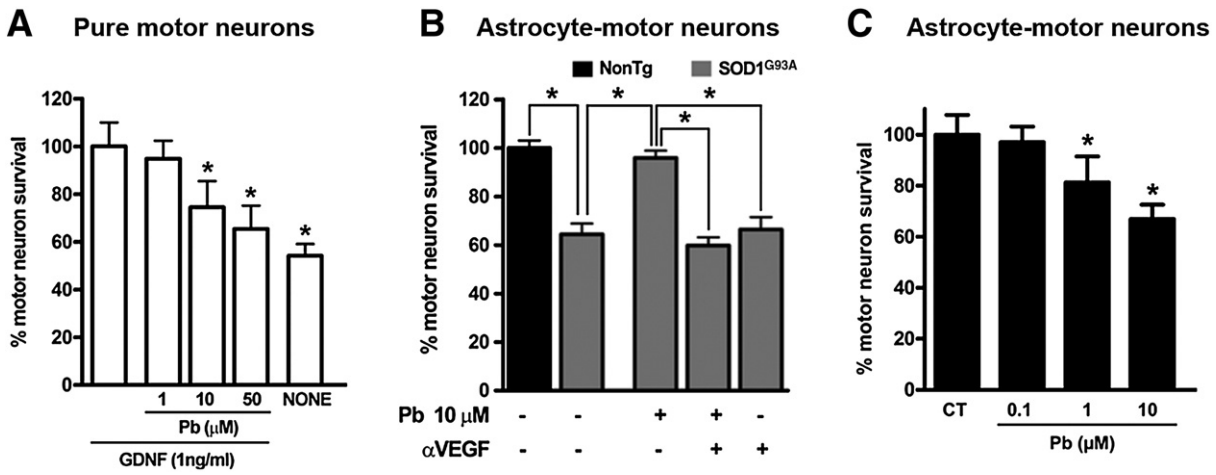

Fig. 5. Effects of Pb-astrocyte exposure on motor neuron survival. (A) Lead toxicity in motor neuron cell culture. Wild type motor neurons were exposed during 48 h to different concentrations of PbAc in the presence of GDNF (1 ng/ml). The dose–response curve revealed significant motor neuron death beginning at 10 μM lead acetate. (*) $p<0.05$: different from control (GDNF). NONE: trophic factor withdrawal. (B) Increased motor neuron survival in transgenic astrocytes pre-exposed to Pb. Astrocyte monolayers were exposed for 24 h to 10 μM of PbAc or vehicle. Wild type motor neurons were plated upon the monolayer after washing out Pb. VEGF-blocking antibody (αVEGF) was added 2 h later to the culture media. Motor neuron survival was analyzed 48 h later. The graph shows the percentage of survival vs. vehicle-treated non-transgenic group (NonTg; black bar). *$p<0.05$: statistically different among the indicated groups. (C) Lead was toxic for motor neurons cocultured on Pb-exposed non-transgenic astrocyte monolayers. Non-transgenic astrocyte monolayers were exposed for 24 h to increasing concentrations of Pb. Media were changed and non-transgenic motor neurons were plated on the top and counted after 48 h. Significant motor neuron death was evident beginning at 1 μM PbAc. *$p<0.05$: different from vehicle-treated cells (CT).

inducing VEGF in SOD1^{G93A} bearing astrocytes. This response was specific for transgenic astrocytes since VEGF levels in non-transgenic astrocytes were below the limits of detection. The proposed mechanism elicited by Pb might explain recent epidemiological data showing that Pb blood and bone levels positively correlate with longer survival in ALS patients after diagnosis (Kamel et al., 2008). Hence, Pb exposure may potentially have opposing actions during the course of ALS, initially promoting the degeneration of motor neurons but later abrogating damage and neuroinflammation mediated by dysfunctional glia (Fig. 6).

Pb blood levels after chronic exposure of low-level Pb in drinking water were comparable with those reported in humans following occupational exposition (Roscoe et al., 2002; Kosnett et al., 2007). These levels did not produce overt Pb toxicity in mice after 90–100 days of treatment. SOD1^{G93A} mice exposed to Pb developed as their littermates receiving vehicle and disease onset was not statistically different. But survival was significantly increased, suggesting a

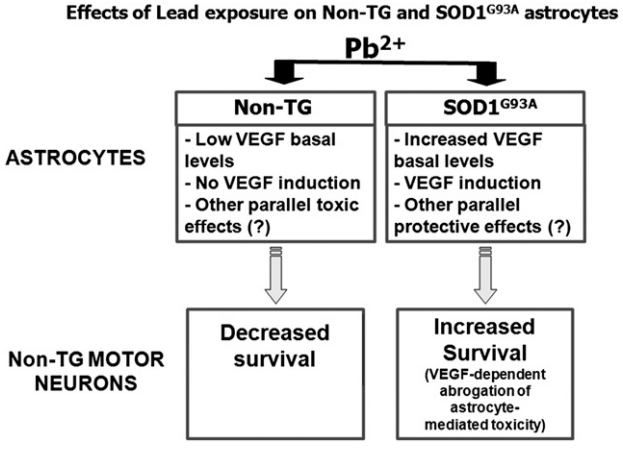

Fig. 6. Proposed mechanisms by which Pb exposure stimulates astrocyte VEGF expression and extends survival in ALS mice. Pb induces VEGF expression specifically in SOD1^{G93A} bearing astrocytes abrogating their neurotoxic phenotype to motor neurons. On the contrary, non-transgenic (Non-TG) astrocytes Pb failed to induce VEGF expression and instead induced a neurotoxic phenotype. Other cytoprotective and/or cytotoxic pathways are likely also stimulated by Pb with yet unknown impact on motor neuron survival.

disease-modifying effect of Pb. Studies in a mouse model of inherited ALS have suggested that onset is related to motor neuron function, while progression depends upon astrocytes and microglia (Boillee et al., 2006; Yamanaka et al., 2008). This is consistent with two pathogenic processes responding differently to Pb intoxication.

Protective Pb effects were evidenced by a dramatic reduction of GFAP-immunoreactive astrocytes, indicating a potential downregulation of ALS-linked neuroinflammation (Levine et al., 1999; Barbeito et al., 2004). Because ALS progression is regulated by neuroglia (Yamanaka et al., 2008), progressive Pb accumulation in astrocytes may reduce their deleterious activation. VEGF administration to SOD1^{G93A} mice also reduced glial reactivity (Zheng et al., 2007), consistent with the concept of reducing neuroinflammation.

Astrocytes survive accumulating Pb to concentrations that are detrimental to neurons (Tiffany-Castiglioni, 1993). In agreement, both cultured SOD1^{G93A} and non-transgenic astrocytes are resistant to moderate Pb concentrations that otherwise are toxic for isolated cultured motor neurons. However, pre-conditioning with Pb caused non-transgenic astrocytes to become neurotoxic to cocultured motor neurons, suggesting that it can also elicit inflammatory-like activation of astrocytes comparable with other stressful stimuli (Cassina et al., 2002). In contrast, motor neurons cocultured on SOD1^{G93A} astrocytes pre-treated with Pb were more likely to survive than on vehicle-treated transgenic astrocytes. These results further suggest that astrocytes bearing the SOD1^{G93A} mutation are regulated differently from non-transgenic astrocytes.

One distinguishing characteristic of Pb-induced VEGF expression is that it specifically occurs in SOD1^{G93A} astrocytes. Non-transgenic astrocytes do not express detectable VEGF levels in response to Pb. VEGF is classically induced by activation of hypoxia-inducible factor-1 (HIF-1) mediated by hypoxic stress and hypoxia-induced generation of reactive oxygen species (Chandel et al., 2000; Xie et al., 2004). However, VEGF expression is also known to be induced by Pb via a PKC/AP1-dependent and HIF-1-independent signaling pathway in an astrocytic cell line (Hossain et al., 2000). Decreased VEGF has been implicated in the pathogenesis of ALS (Oosthuyse et al., 2001; Lambrechts et al., 2003; Storkebaum et al., 2005; Wang et al., 2007). VEGF protects motor neurons in culture and in organotypic cultures (Li et al., 2003; Van den Bosch et al., 2004; Tolosa et al., 2008). Furthermore, VEGF was shown to be released by astrocytes (Van den Bosch et al., 2004) and exert a potent trophic activity for motor

neurons by activating VEGFR-2 (Storkebaum et al., 2005). Interestingly, our results indicate that Pb increased VEGF expression in the spinal cord of SOD1^{G93A} mice. Basal VEGF expression was greater in untreated SOD1^{G93A} mice compared to non-transgenic controls as previously observed (Murakami et al., 2003). Nevertheless, the protection is appreciated when basal levels are increased suggesting that additional protective mechanisms may be enhancing VEGF release or bioavailability. Additionally, Pb may modulate ALS pathogenesis by affecting other physiological mechanisms. For example, it has been shown that Pb may decrease SOD activity in lead-exposed rats (Babu et al., 2007), which could have beneficial effects on familiar ALS models. Furthermore, at the cellular level, Pb potently stimulates the antioxidant enzyme hemoxygenase-1 expression in astrocytes (Cabell et al., 2004), suggesting increased antioxidant defenses and cytoprotective pathways that may also contribute to neuronal protection.

Understanding how Pb stimulates VEGF production in non-neuronal cells by the ALS disease process can reveal a simpler therapeutic route than gene therapy approaches under current development.

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