Stabilization of Hyperdynamic Microtubules Is Neuroprotective in Amyotrophic Lateral Sclerosis

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Mutations in copper/zinc superoxide dismutase 1 (SOD1), a genetic cause of human amyotrophic lateral sclerosis, trigger motoneuron death through unknown toxic mechanisms. We report that transgenic SOD1G93A mice exhibit striking and progressive changes in neuronal microtubule dynamics from an early age, associated with impaired axonal transport. Pharmacologic administration of a microtubule-modulating agent alone or in combination with a neuroprotective drug to symptomatic SOD1G93A mice reduced microtubule turnover, preserved spinal cord neurons, normalized axonal transport kinetics, and delayed the onset of symptoms, while prolonging life by up to 26%. The degree of reduction of microtubule turnover was highly predictive of clinical responses to different treatments. These data are consistent with the hypothesis that hyperdynamic microtubules impair axonal transport and accelerate motor neuron degeneration in amyotrophic lateral sclerosis. Measurement of microtubule dynamics in vivo provides a sensitive biomarker of disease activity and therapeutic response and represents a new pharmacologic target in neurodegenerative disorders.

Amyotrophic lateral sclerosis (ALS) is a late-onset, progressive neurodegenerative disease affecting motoneurons (1). The etiology of the majority of ALS cases is unknown, but ~20% of familial disease cases are due to mutations in copper–zinc superoxide dismutase1 (SOD1) (2). This led to the development of SOD1 transgenic mice as models of disease (2–5).

Physically, motoneurons are unique, representing the longest cells in the body, with axons of some motoneurons in the spinal cord extending a meter or more to reach an end organ. As a result of this morphology, exceptional demands are placed on motoneurons. Active transport along lengthy axons is required to convey newly made materials from the cell body to the farthest nerve endings, and to convey nutrients and metabolites back to the cell body. Microtubules are an essential component of the neuron’s scaffold and represent the “roadway,” or conveyer belt, that neurons use to transport nutrients (6–10).

Microtubule-based transport is mandatory for survival of motoneurons and muscle cells; changes in slow axonal transport have been linked to neuropathogenesis in mutant SOD1 transgenic mice (11–13). In addition, the assembly and disassembly of microtubule polymers in motoneurons is highly responsive to cellular insults, such as excitotoxic stimuli (8, 14–16).

The relation between dynamics of microtubules and neuronal pathogenesis has not been explored in detail, in part due to limited techniques for measuring microtubule dynamics in vivo. In most non-neuronal cells, tubulin dimers and microtubule polymers exist in rapid dynamic equilibrium, as we have recently shown in vivo by isotopic labeling (17). In neurons, however, this rapid turnover of axonal and dendritic microtubules is believed to be less dynamic due to their interactions with a specific subclass of microtubule-associated proteins (MAPs) (18–20). This stability of microtubules is presumed to be necessary to maintain the integrity of the microtubule-based axonal transport of synaptic vesicles and nutrients (21, 22). Accordingly, we hypothesized that instability of microtubules, i.e. the induction of a population of hyperdynamic microtubules, whether due to altered MAP function, intrinsic factors in the microtubules, or excitotoxic stimuli, might compromise axonal transport and represent a novel pathogenic mechanism in neurodegenerative disorders. This could then provide new therapeutic targets in motoneuron diseases, including ALS.

We recently developed a stable isotope-mass spectrometric technique for quantifying the turnover kinetics of specific microtubule populations in vivo (17). Here, we apply this technique to neurons and test the above hypothesis. We report that microtubule dynamics are abnormal in the SOD1G93A transgenic mouse model of ALS, in association with altered axonal transport. Moreover, pharmacologic interventions that reduce the fraction of hyperdynamic microtubules prevent neuronal death, restore axonal transport, delay the onset of disease, and extend survival in the SOD1G93A mouse.
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EXPERIMENTAL PROCEDURES

Genetic Background of SOD1G93A Mice, Disease Onset, and Survival—All experiments received approval from the local animal use committee and were carried out according to Office of Laboratory Animal Welfare-National Institutes of Health guidelines. Transgenic mice (female and male) carrying human SOD1 gene with a G93A mutation (TgN [SOD1-G93A] 1Gur) were obtained from The Jackson Laboratory or were donated from Project A.L.S. The colony was maintained by breeding male heterozygous carriers to female B6SJLF1 hybrids. Animals were housed in a controlled temperature and humidity environment and maintained on a 12-h light/dark cycle, with access to food and water provided ad libitum. Mice were assessed daily for survival and bi-weekly for weighing and stride length analysis starting at 10 weeks of age. The disease end point was determined as previously described (4, 23–25) based on asymmetrical or symmetrical paralysis of the hind limbs and the inability to right themselves within 15 s after being placed on their side.

Animal Studies—Each experimental group contained age-matched drug-treated and untreated littermates. All experiments were performed blinded with coded drugs. Noscapine (an microtubule-modulating agent (MTMA)) was obtained from Sigma, and pioglitazone (Pio) and riluzole were from an microtubule-modulating agent (MTMA)). Animals were intraperitoneally injected with 30–35 ml/kg 2H2O (99.9 mol% 2H2O) from Sigma, which was previously shown to have two components, based on rates of transport have been linked to neuropathogenesis in mutant SOD1 transgenic mice (11–13). Slow axonal transport has previously been shown to have two components, based on rates of movement of cargo: one at ~0.5 mm/day (SCa) and the other at ~1–2 mm/day (SCb). Both components include the transport of tubulin (13). Using a stable isotope-mass spectrometric technique, we measured the in vivo rate of transport of endogenously 2H-labeled tubulin in sciatic nerves of SOD1G93A mice (Fig. 1). Tubulin is synthesized in the cell body and transported along the axon. To include all forms of transport, whether as individual dimers, oligomers, or polymers (7, 27), we measured the kinetics of label incorporation into combined sol-verse sections were cut and stained with galloccyanin (Nissl-stain). Nissl-stained motoneurons located within the sciatic motor pool, in which the nucleolus was clearly visible, were counted in each ventral horn on every third section between the L2 and L5 levels of the spinal cord.

Processing of Tubulin for GC/MS Analysis—Tubulin samples from MTs or dimers were hydrolyzed by treatment with 6 N HCl for 16 h at 110 °C. Protein-derived amino acids were derivatized to pentafluorobenzyl derivatives, and 2H incorporation in alanine released from tubulin was measured by GC/MS, as described elsewhere (17, 26). 2H enrichment was calculated as the increase, over natural abundance, in the percentage of alanine present as the (M+1) mass isotope (EM1). The processing and derivatization procedures for amino acid analysis remove all exchangeable hydrogen atoms (e.g. -OH and -NH bonds), so that only the 2H label incorporated into stable -CH bonds in vivo is measured (17, 26).

Measurement of 2H2O Enrichment in Body Water—Measurement of 2H2O enrichment in body water was measured using a modification of previously described procedures (17). Briefly, hydrogen atoms from plasma water were transferred to acetylene by reaction with calcium carbide. Acetylene samples were then analyzed using a Series 3000 cycloidal mass spectrometer (Monitor Instruments), which was modified to record ions at m/z 26 and 27 (M0 and M1) and calibrated against a standard curve prepared by mixing 99.9% 2H2O with unlabeled water. Body water 2H enrichments were not affected by drug treatment (data not shown).

Statistical Analysis—The fraction of newly synthesized alanine in each sample was calculated as the ratio of the measured EM1 value to the maximal or asymptotic value possible at the measured body water enrichment, calculated by mass isotope distribution analysis, as described elsewhere (26). This ratio represents the fraction of tubulin that was newly synthesized. The presence of newly synthesized tubulin in MTs represents the assembly or dynamics of polymer from free tubulin dimers during the period of label exposure. The statistical significance of drug or treatment effects was assessed by one-way analysis of variance with Tukey post-hoc testing. Kaplan-Meier survival analysis was used for survival comparisons. Software for statistics included SigmaStat 3.0 and Microsoft Excel 2003.

RESULTS

Hyperdynamic Microtubules Accompany Impaired Axonal Transport and Disease Progression—Changes in slow axonal transport have been linked to neuropathogenesis in mutant SOD1 transgenic mice (11–13). Slow axonal transport has previously been shown to have two components, based on rates of movement of cargo: one at ~0.5 mm/day (SCa) the other at ~1–2 mm/day (SCb). Both components include the transport of tubulin (13). Using a stable isotope-mass spectrometric technique, we measured the in vivo rate of transport of endogenously 2H-labeled tubulin in sciatic nerves of SOD1G93A mice (Fig. 1). Tubulin is synthesized in the cell body and transported along the axon. To include all forms of transport, whether as individual dimers, oligomers, or polymers (7, 27), we measured the kinetics of label incorporation into combined sol-
ubulin dimers and polymers in serial anatomical segments of the sciatic nerve.

An intraperitoneal bolus of 30–35 ml/kg $^2$H$_2$O (99.9 mol% $^2$H$_2$O) was administered to 10-week-old wild-type (WT) and SOD1G93A transgenic mice, resulting in 4–5% body water $^2$H enrichment that washed out over the next 4–5 days (26). At 21 days post-labeling (13 weeks of age), the L5 roots and sciatic nerves were removed and cut into 2-mm segments (Fig. 1A). The soluble tubulin dimer and microtubule polymer fractions (Fig. 1B) were separated by centrifugation and combined for analysis by GC/MS for $^2$H isotope enrichment of tubulin-bound amino acids (17, 26). After 21 days, $^2$H-labeled tubulin had advanced well along the nerve of WT mice, revealing a clear dual wave pattern (Fig. 1A). The SCa fraction moved at ~0.3 mm per day, and the SCb fraction moved at ~0.7 mm per day, similar to published values (13). In the WT mice, slightly more labeled tubulin was present in the SCa fraction after 3 weeks compared with age-matched WT littermates (n = 3, mean ± S.D.).

To test whether alterations in microtubule dynamics underlies impaired axonal transport $^2$H$_2$O (8%) in drinking water was administered to WT and SOD1G93A transgenic mice and microtubule polymerization/depolymerization (dynamics) rates were measured as described previously (17). The $^2$H$_2$O administration was begun at 8.5, 11, 12.5, and 16 weeks of age, and animals were sacrificed after 48 h of labeling. The lumbar region of the spinal cord (between L2 and L5 levels) and the whole length of both sciatic nerves were dissected and carefully removed. Cytosolic extracts were fractionated to isolate free tubulin dimers and microtubule polymers (Fig. 1B). After separation, microtubule polymers (MTs) were further isolated as dendritic (i.e. MAP2-associated MTs) and dynamic axonal MTs (i.e. Tau-associated MTs) by making use of the compartment-specific distribution of the neuronal MAPs Tau and MAP2 (28–32). This was achieved by sequential binding to immunoprecipitation Tau5 and MAP2 columns, leaving all other microtubules in the unbound fraction (Fig. 1B and see supplemental “Methods”). It should be noted that Tau5 and MAP2 antibodies recognize their antigens independently of phosphorylation state and variable exons of MTs, thereby binding to all isoforms (29, 32). The vast majority of MAP2 and Tau-non-associated fractions are the cold-stable (CS) microtubules (28, 30, 31).

As judged by Western blotting, Tau-associated MTs were quantitatively captured by the Tau5 affinity column (see supplemental Fig. 6A, lane 3). MAP2-associated MTs were found in the flow-through (see supplemental Fig. 6A, lane 2), confirming the specificity of the affinity separation and indicating that microtubules associated with both MAPs were rare or absent. Tubulin was purified from each fraction to ≥95% purity as determined by SDS-PAGE (see supplemental Fig. 6B).

Altered dynamics of Tau-associated and CS MTs was detected in SOD1G93A mice as early as 8.5 weeks of age (presymptomatic), and MT dynamics increased as the mice approached disease onset and began to show symptoms (Fig. 1C). The CS MTs (enriched for axonal shaft) isolated from sciatic nerve of WT mice were the least dynamic population and remained so in older WT mice. In contrast, CS MTs from sciatic nerve of SOD1G93A mice were strikingly more dynamic at 8.5 weeks, which increased with age. The total content of Tau-associated and CS MTs in sciatic nerve of SOD1G93A mice was indistinguishable from WT littermates, indicating that greater label incorporation in MT represented true turnover rather than net assembly (de novo formation) of MT (see supplemental Fig. 7A).

A similar degree of hyperdynamicity was observed in all the populations of MTs isolated from sciatic nerve, cerebral cortex,
and lumbar region of the spinal cord of symptomatic (13 weeks old) SOD1G93A mice (Fig. 2A). Cortical neurons as well as the lumbar spinal cord neurons showed particularly increased turnover of CS MTs. This increase was ~5-fold in the cerebral cortex and the lumbar region of the spinal cord and over 8-fold in sciatic nerve, compared with WT mice (Fig. 2A). With the exception of Tau-MTs from the cerebral cortex and MAP2-MTs from the cervical region of the spinal cord, all the other Tau- and MAP2-associated MTs showed higher dynamic in G93ASOD1 mice at the symptomatic age of 13 weeks compared with WT mice (Fig. 2A). Thus, the spatial distribution of MT hyperdynamicity corresponds to established areas of neuronal degeneration in symptomatic SOD1G93A mice, which exhibit acute axonal pathology along the corticospinal tract and sciatic nerve (4, 5).

Importantly, label incorporation measured in the different MT subsets does not reflect exchange with tubulin dimers in vitro during extraction and purification. No label incorporation into Tau- or MAP2-associated MTs was detected when purified ²H-labeled tubulin dimers were added to unlabeled spinal cord extracts (see supplemental Fig. 7B). ²H incorporation into

MTs therefore represents in vivo incorporation of labeled tubulin dimers that were newly synthesized during the period of ²H₂O exposure (17, 26).

Because the sciatic nerve contains both motor and sensory fibers, we compared MT dynamics in a motor nerve (rectus femoris muscle) and a sensory nerve (saphenous nerve) (Fig. 2B). After 48 h of ²H₂O labeling, the Tau-associated and CS MTs from the motor nerve were extremely hyperdynamic in symptomatic SOD1G93A mice (13 weeks), whereas no perturbation was observed in the same MT populations from the sensory nerve (Fig. 2B). Taken together, these results reveal the presence of markedly hyperdynamic MT subpopulations in the peripheral motor and sciatic mixed nerves, in the cerebral cortex, and in the lumbar region of the spinal cord, but not in sensory nerves, of SOD1G93A mice. Furthermore, hyperdynamicity of MTs occurs early in disease progression and correlates with impaired axonal transport of nutrients.

Stabilization of Hyperdynamic Microtubules Delays Disease Onset and Increases Survival—Perturbation of MT dynamics appears to be an early event in motoneuron degeneration in SOD1G93A mice. Based on these findings, we determined whether pharmacologic modulation of MT dynamics prevents neuronal dysfunction and results in clinical benefits.

We first examined the effect on MT dynamics of a specific MTMA, noscapine (33, 34), and compared with two drugs known to have activity in the treatment of ALS, riluzole, and the anti-inflammatory peroxisome proliferator-activated receptor-γ agonist Pio (24, 25, 35). All drugs were administered to symptomatic SOD1G93A mice beginning at 10 weeks of age. Treatment was for 3 weeks, and ²H₂O (8%) was administered during the last 2 days of treatment. The lumbar region of the spinal cord and the entire length of both sciatic nerves were dissected and removed. As shown in Fig. 3A, both MTMA and Pio decreased but did not normalize MT dynamics, whereas riluzole did not have significant general effects but modulated only the Tau- and MAP2-associated MTs in the lumbar region of the spinal cord.

Next, we investigated whether co-administration of MTMA/riluzole or MTMA/Pio would further reduce hyperdynamicity of MTs (Fig. 3B). The same treatment and labeling protocol was used, with combination drug therapy started at 10 weeks. Both treatment regimens reduced turnover of MTs, with the MTMA/Pio combination restoring MT dynamics much closer to levels observed in aged-matched WT mice than the MTMA/riluzole combination (Fig. 3B). The effect of MTMA/Pio was detected in all MT compartments of both the lumbar region of the spinal cord and the sciatic nerve.

We then evaluated whether pharmacologic modulation of hyperdynamic MTs results in clinical benefits in SOD1G93A mice and whether the degree of MT stabilization predicted clinical outcomes. Motor performance and survival were assessed in groups of 20 animals. No significant differences were observed between mixed gender and single-gender (female) groups of untreated and treated SOD1G93A mice (see supplemental Fig. 8, A and B). The groups consisted of 10 male and 10 female mice, except that 20 females were used for MTMA/Pio, due to a scarcity of males.
Untreated SOD1G93A mice exhibited an age-dependent decline in motor performance, as determined by stride length measurements (Fig. 3). The onset of disease in SOD1G93A mice (defined as a 40% reduction in stride length) was observed at day 90 followed by a rapid decline that progressed to a stage of complete hind limb paralysis and a disease end point of 121 days (mean ± S.D.; n = 20, mixed gender; Fig. 3C). Riluzole-treated mice experienced no significant delay in disease onset or subsequent rate of decline in stride-length performance (Fig. 3C).

SOD1G93A mice treated with the two-agent combination, MTMA/Pio, exhibited delayed onset of disease (Fig. 3C and supplemental Fig. 8C), and better stride-length performance between 80.5 and 120 days of age, compared with untreated SOD1G93A mice (Fig. 3C and supplemental Fig. 8C). The MTMA/Pio-treated mice (mean ± S.D., n = 20 female) also experienced a delayed decline in body weight (supplemental Fig. 9A) and significant increase in survival from 118.5 ± 4.2 days (untreated) to 149.7 ± 7.1 days (31.2 days, 26% increase, p < 0.001, Fig. 4A and supplemental Fig. 8D). The MTMA/
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TABLE 1
Correlation between MT dynamics and survival in SOD1G93A mice

| Treatment          | MT modulation | Survival | Increase lifespan |
|--------------------|---------------|----------|-------------------|
| Untreated (♂/♀)    | N/A           | 121.0 ± 5.8 | N/A              |
| Riluzole (♂/♀)     | 55            | 126.05 ± 5.7 | 4                |
| Pio (♂/♀)          | 70            | 134.5 ± 6.2  | 11               |
| MTMA (♂/♀)         | 75            | 137.9 ± 6.4  | 14               |
| MTMA/riluzole (♂/♀)| 78            | 140.5 ± 6.9  | 16               |
| MTMA/Pio (♀)       | N/A           | 118.5 ± 4.2  | N/A              |
| MTMA/riluzole (♀)  | 78            | 135.5 ± 4.7  | 14               |
| MTMA/Pio (♀)       | 85            | 149.7 ± 7.1  | 26               |

* N/A, not applicable.

3-week treatment with MTMA alone partially normalized and MTMA/Pio completely restored the transport of [3H]tubulin in both ScA and ScB fractions (Fig. 5B). Interestingly, treatment with the combination of MTMA/riluzole had modest effects on both the transport of [3H]tubulin and on hyperdynamic MTs in sciatic nerve (non-significant change for Tau-MTs and ~25% reduction for CS-MTs, Fig. 3B). These results demonstrate that modulation of abnormal microtubule dynamics is generally associated with restored axonal transport and increased motoneuron survival in SOD1G93A mice.

DISCUSSION

The dynamics or turnover of MTs (dynamic exchange between MT polymers and free tubulin dimers) had not previously been related to motoneuron degeneration. Direct observations of neuronal MT dynamics have been limited to a few studies in cell culture, tracking the incorporation of microinjected, fluorescently tagged tubulin into MTs, or measuring fluorescence recovery after photobleaching (36–38). The stable isotope-mass spectrometric approach described here represents the first direct exploration of neuronal MT dynamics in vivo. The availability of this tool permitted us to evaluate MT dynamics in SOD1G93A mice, which remained altered when compared with WT littermates (Fig. 5, A and B). In contrast, riluzole-treated mice experienced delay in disease onset between 80.5 and 115.5 days of age (Fig. 3C and supplemental Fig. 8A) and in the decline of body weight (see supplemental Fig. 9B), with a 16% increase in lifespan (to 140.5 ± 6.9 days, n = 20 mixed gender, p < 0.001, Fig. 4A). Thus, the increase in lifespan was between 4 and 26% (Table 1), and there was a highly significant correlation between the biochemical measure of MT dynamics in vivo at 13 week of age, after 3 weeks of treatment, and both clinical outcomes, motor function and survival, with long-term treatment (Fig. 4B and Table 1).

Stabilization of Hyperdynamic Microtubules Preserves Motoneurons and Rescues Axonal Transport—We next evaluated whether the potent pharmacologic modulation of hyperdynamic MTs observed in response to two-agent combinations promotes survival of motoneurons and rescues impaired axonal transport, as predicted by our hypothesis that microtubule instability compromises axonal transport and represents a mechanism of neurodegeneration.

The number of motoneurons was counted in a segment of the sciatic motor pool of each spinal cord. The effect was assessed after 5 weeks of treatment, in 15-week-old SOD1G93A mice, to allow significant loss of motoneurons. Examples of Nissl-stained spinal cord sections of WT controls, untreated, MTMA/riluzole-treated, and MTMA/Pio-treated SOD1G93A mice are shown (Fig. 4C, panels 1–4). A significant loss of motoneurons in the sciatic pool was observed in untreated mice (Fig. 4C, panel 2 and D), with 121.0 ± 5.8 motoneurons counted compared with 126.05 ± 5.7 in WT littermates (Fig. 4D). Treatment with MTMA/Pio largely prevented loss of motoneurons, with 118.5 ± 4.2 motoneurons counted (p < 0.05 versus untreated, Fig. 4D). In contrast, no significant preservation of the number of motoneurons surviving was observed in the MTMA/riluzole-treated mice (121.0 ± 5.8, Fig. 4D). Five weeks of treatment with MTMA/riluzole and MTMA/Pio showed similar effects on hyperdynamic MTs in the lumbar region of the spinal cord (see supplemental Fig. 10).
dynamics as a potential neuropathogenic factor, therapeutic target, and biomarker in axonal degeneration. The underlying biologic hypothesis was that transport of materials and nutrients along MTs is impaired if the “road is constantly under construction”; i.e. if the assembly/disassembly cycle is occurring at an abnormally high rate, even in the absence of apparent ultrastructural abnormality in the MT array. Abnormal accumulation of tubulin dimers in the cell body and the proximal axonal segment and altered levels of MAPs have been reported in the spinal motor neurons of SOD1G37R and SOD1G93A mice, as well as in ALS patients (39–43). Changes in slow axonal transport have also been linked to disease pathogenesis in mutant SOD1 transgenic mice (12, 13). We confirmed altered axonal transport in SOD1G93A mice, by using a novel stable isotope labeling approach, and also found dramatically altered MT turnover (hyperdynamic MTs) in spinal motoneurons and sciatic nerve, which was evident well before the onset of symptoms and increased as disease progressed. More importantly, targeted pharmacologic reduction of hyperdynamic MTs resulted in recovery of axonal transport, increased motoneuron survival, significant delays in symptoms, and a greater degree of life extension (up to 26%) than had previously been reported in these mice. Results were highly reproducible in a large number of mice (n = 20) in each treatment arm.

The MTMA, noscapine, was chosen for these studies because of its particular actions. Noscapine is known to bind specifically and stoichiometrically to tubulin (33). Unlike other microtubule-stabilizing drugs, such as paclitaxel or other taxanes, noscapine does not significantly promote microtubule polymerization and does not alter the tubulin polymer/monomer ratio. Instead, noscapine modulates microtubule dynamics by reducing the growing and shortening rates and increasing the percentage of time that the microtubules spend in the attenuated state (neither growing nor shortening detectably), thus reducing the overall dynamicity of the microtubules (33, 34). Testing an MT-modulating agent rather than an MT-stabilizing agent was a key element in our strategy. MT-stabilizing agents, like paclitaxel, have been shown to alter the tubulin polymer/monomer ratio and bundle the resulting MTs, leading to limb motor deficits and peripheral neuropathy in the murine melanoma model and patients (17, 44). There is also evidence that treatment with paclitaxel induces detachment of Tau from MTs in cells (45). The pharmacologic strategy tested here was therefore to temper the hyperdynamicity of MTs rather than to change MT polymer mass or alter the structural features of MTs.

The finding that an agent known to have anti-inflammatory and anti-oxidative activities, pioglitazone (25, 35), reduced MT hyperdynamicity in SOD1G93A mice, may be explained mechanistically through a reduction in excitotoxic or inflammatory stimuli. These stimuli have been shown to alter ex vivo MT dynamics in neurons (8, 14, 46, 47). Although we did not measure inflammatory mediators, it has been reported that pro-inflammatory cytokines and inducible nitric-oxide synthase are up-regulated prior to motoneuron death in the lumbar spinal cord of SOD1G93A mice (35). Moreover, application of nitric oxide (NO) to cultured chick sensory neurons has also been shown to alter MT configurations and induce axonal retraction (47). Pioglitazone reduces inducible nitric-oxide synthase induction and NO-mediated toxicity, while ameliorating the progression of disease in SOD1G93A mice (35). These results suggest that pioglitazone could modulate MT dynamics by down-regulating pro-inflammatory cytokines and inducible nitric-oxide synthase.

An interesting implication is that hyperdynamic MTs may participate in a positive feedback loop whereby a primary neuronal insult can impair axonal transport and further exacerbate cellular injury. Normalization of MT dynamics may interfere with this vicious cycle and thereby provide benefits either as a primary or a secondary therapeutic target. Late or terminal pathogenic events appear to be difficult to slow by this treatment approach, however, as evidenced by the similar slopes of the age of death curves (Fig. 4) and the terminal phase of the stride-length curves (Fig. 3C). Microtubule dynamics were also abnormal at end-stage in treated animals (data not shown), consistent with escape from treatment effects.

In summary, several aspects of these findings deserve emphasis. First, altered MT dynamics in motoneurons may be a fundamental feature of the biology of ALS. More generally, the maintenance of balanced MT dynamics can be seen as an “Achilles heel” of neuronal survival, particularly for large caliber motoneurons, which are highly dependent on efficient axonal transport of nutrients. Second, altered MT dynamics is evident long before the onset of clinical symptoms and predicts motoneuron degeneration in the SOD1G93A transgenic mouse model of ALS, representing a potential biomarker of disease activity. Finally, pharmacologic modulation of MT hyperdynamicity represents a novel strategy for restoring axonal transport and protecting neurons. Because the cellular and biochemical phenotypes of sporadic and familial ALS overlap considerably, we anticipate that modulation of pathologic MT dynamics is likely to be beneficial for both types of ALS. In the absence, however, of an animal model for sporadic ALS, this will have to be resolved by human studies.

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