

Additive neuroprotective effects of creatine and cyclooxygenase 2 inhibitors in a transgenic mouse model of amyotrophic lateral sclerosis

Peter Klivenyi,¹ Mahmoud Kiaei,¹ Gabrielle Gardian, Noel Y. Calingasan and M. Flint Beal

Department of Neurology and Neuroscience, New York Presbyterian Hospital–Weill Medical College of Cornell University, New York, New York, USA

Abstract

There is substantial evidence implicating both inflammation and mitochondrial dysfunction in amyotrophic lateral sclerosis (ALS) pathogenesis. We investigated the therapeutic effects of cyclooxygenase 2 (COX-2) inhibitors both alone and in combination with creatine in the G93A transgenic mouse model of ALS. Oral administration of either celecoxib or rofecoxib significantly improved motor performance, attenuated weight loss and extended survival. The administration of COX-2 inhibitors significantly reduced prostaglandin E2 levels at 110 days of age. The combination of creatine with COX-2

inhibitors produced additive neuroprotective effects and extended survival by approximately 30%. The COX-2 inhibitors significantly protected against depletion of anterior horn motor neurons and creatine with COX-2 inhibitors showed greater protection than COX-2 inhibitors alone. These results suggest that combinations of therapies targeting different disease mechanisms may be a useful strategy in the treatment of ALS.

Keywords: creatine, cyclooxygenase, free radicals, mitochondria, prostaglandin E2, superoxide dismutase. *J. Neurochem.* (2004) **88**, 576–582.

Amyotrophic lateral sclerosis (ALS) is one of the most common adult-onset neurodegenerative diseases. A major advance in studying the disease pathogenesis was the discovery of mis-sense mutations in the gene coding for Cu/Zn superoxide dismutase 1 (SOD1) in a subset of patients with autosomal dominant inherited ALS (Rosen *et al.* 1993). This led to the development of transgenic mouse models of ALS, which has spurred research into pathogenetic mechanisms (Gurney 1994; Wong *et al.* 1995). Elucidating the mechanism by which the SOD1 mutations lead to disease remains challenging. Nevertheless, the transgenic mouse models have suggested that both mitochondrial dysfunction and inflammatory mechanisms contribute to disease pathogenesis. Mitochondrial swelling and vacuolization are amongst the earliest pathological features found in two strains of transgenic ALS mice with SOD1 mutations (Wong *et al.* 1995; Gurney *et al.* 1998). An increase in cyclooxygenase 2 (COX-2) message, protein and prostaglandin E2 (PGE2) levels was found in both transgenic ALS mice as well as in post-mortem brain tissue, cerebrospinal fluid and serum from sporadic ALS patients (Almer *et al.* 2001, 2002; Ilzecka 2003).

We previously showed that creatine administration, which can compensate for mitochondrial dysfunction, exerts neuroprotective effects and extends survival in transgenic mouse models of ALS (Klivenyi *et al.* 1999). Others have shown that COX-2 inhibitors protect motor neurons *in vitro* and extend survival or delay disease onset in transgenic mouse models of ALS (Drachman and Rothstein 2000; Drachman *et al.* 2002; Pompl *et al.* 2003). In the present study we examined whether creatine could exert additive neuroprotective effects when administered with either celecoxib or rofecoxib, the two COX-2 inhibitors presently approved for use in man.

Received August 13, 2003; revised manuscript received September 22, 2003; accepted September 23, 2003.

Address correspondence and reprint requests to M. Flint Beal, MD, Department of Neurology and Neuroscience, New York Presbyterian Hospital–Weill Medical College of Cornell University, 525 East 68th Street, New York, NY 10021, USA. E-mail: fbeal@med.cornell.edu

¹These authors contributed equally to this manuscript.

Abbreviations used: ALS, amyotrophic lateral sclerosis; COX-2, cyclooxygenase 2; IL, interleukin; PGE2, prostaglandin E2; SOD1, superoxide dismutase 1.

Materials and methods

Mice

Transgenic mice with the G93A mutation of the human SOD1 gene (G1H/1) mutation [B6SJL-TgN (SOD1-G93A) 1 Gur] were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and were bred locally with female B6SJL mice (Jackson Laboratories). The offspring were genotyped by PCR assay of DNA obtained from tail tissue. The mice were fed either a diet supplemented with 2% creatine (Avicena, Inc., Cambridge, MA, USA), 0.005% rofecoxib (Vioxx, Merck & Co., Inc., Whitehouse Station, NJ, USA), 0.012% celecoxib (Celebrex; Pfizer, Inc., New York, NY, USA) or the combination of 2% creatine and rofecoxib or celecoxib, starting at 30 days of age. Twelve mice fed unsupplemented diets served as a control. The number of G93A mice in each treatment group was 11–13. An additional six to seven mice in each group were studied histologically. The experimental groups were balanced by sex. All animal experiments were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the local animal care committee.

Behavioral testing (rotorod)

Mice were given 5 days to become acquainted with the rotorod apparatus (Columbus Instruments, Columbus, OH, USA). Testing then began with the mice trying to stay on a rod that was rotating at 12 r.p.m. and the time when they fell off the rod was used as the measure of competence on this task. The maximum score was 300 s, each mouse was given two trials and the best result was recorded. Mice were tested every other day until they could no longer perform the task. The mice were weighed once a week.

Survival

G93A transgenic mice initially showed a resting tremor that progressed to gait abnormalities, paralysis of the hindlimbs, then to paralysis of the forelimbs and finally to complete paralysis. Mice were killed when they could no longer roll over within 10 s of being pushed on their side. This time point was used as the time of death.

Prostaglandin E2 tissue content

The levels of PGE2 in brain and spinal cord tissues were measured by enzyme-linked immunoassay kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. Briefly, frozen cerebral cortex and spinal cords were homogenized in 0.1 M phosphate-buffered saline (containing 1 mM EDTA and 10 μ M indomethacin). After homogenization, samples were spiked with 10 000 d.p.m. of 5,6,8,11,12,14,15(n)[³H] PGE2 (specific activity 151 Ci/mmol; Amersham, Piscataway, NJ, USA), mixed and centrifuged at 10 000 g for 25 min. Supernatant fluids were removed, mixed with an equal volume of ethanol and centrifuged at 1500 g. The supernatant fluids were diluted with 50 mM acetate buffer and purified through an affinity column (Cayman Chemical) according to the manufacturer's instructions. The purified samples were evaporated, redissolved in enzyme-linked immunoassay buffer and applied to 96-well plates pre-coated with goat anti-mouse IgG and incubated with PGE2 monoclonal antibody and recovery tracer for 18 h at 4°C. The plate was rinsed five times with washing buffer and developed using Ellman's reagent for 60–90 min at room temperature with gentle shaking. The PGE2-specific concentration

was determined spectrophotometrically and calculated by plotting the standard percentage B/B0 (% sample or standard Bound/Maximum Bound) versus PGE2 concentration (in pg/mL) using PGE2 analysis software and expressed in pg/mg of tissue.

Histological evaluation

G93A transgenic mice and N1029 mice (110 days old, $n = 6$ or 7 per group) were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and perfused transcardially with ice-cold 0.9% NaCl followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The spinal cords were removed, post-fixed for 2 h in the same fixative and then cryoprotected in 30% sucrose overnight at 4°C. Serial-cut coronal tissue sections (50 μ m thick) from lumbar 1–4 segments were cut using a cryostat and mounted onto gelatin-coated slides. The sections were stained for Nissl substance with cresyl violet and examined under a Nikon Eclipse E600 microscope (Morrell Instruments, Melville, NY, USA). The total number of Nissl-stained neurons was estimated within the ventral horn of 20 serial-cut tissue sections using the optical fractionator method in the Stereo Investigator (version 4.35) software program (Microbrightfield, Burlington, VT, USA). Cell counts were made within an area demarcated by a horizontal line drawn through the central canal and encompassing the ventral horn of the gray matter to include layers 7–9. Data are expressed as the mean \pm SEM. Immunocytochemistry was carried out with a COX-2 specific antibody (Cayman Chemical).

Statistical analysis

Statistical comparisons were by one-way ANOVA or by repeated measures ANOVA followed by Newman–Keuls post-hoc test.

Results

Oral administration of 2% creatine, 0.005% rofecoxib and 0.012% celecoxib in the diet resulted in significant improvements in the survival of G93A mice compared with the survival of mice fed an unsupplemented diet (Fig. 1). The mean survival of mice on supplemented diets increased from 126.1 ± 2.6 to 151.4 ± 2.1 days with creatine ($p < 0.0001$), to 152.3 ± 4.2 days with rofecoxib ($p < 0.0001$), to 150.2 ± 4.4 days with celecoxib ($p < 0.0001$), to 162.1 ± 4.0 days with creatine/celecoxib ($p < 0.0001$) and to 165.1 ± 4.2 days with creatine/rofecoxib ($p < 0.0001$). An additive neuroprotective effect was seen in the combined groups compared with the creatine-, celecoxib- and rofecoxib-treated groups ($p < 0.05$). Survival was extended by 25 days with creatine, 26 days with rofecoxib, 24 days with celecoxib, 36 days with the creatine/celecoxib combination and 39 days with the creatine/rofecoxib combination.

The treated mice had significantly better motor performance from 99 to 134 days of age than mice fed unsupplemented diets (Fig. 2). Additive improvement was seen in the combination groups of creatine/celecoxib or creatine/rofecoxib as compared with creatine, celecoxib and rofecoxib alone. The COX-2 inhibitors and creatine also delayed weight loss, shown by a significantly higher weight in the treated animals from 115 days of age. All compounds and

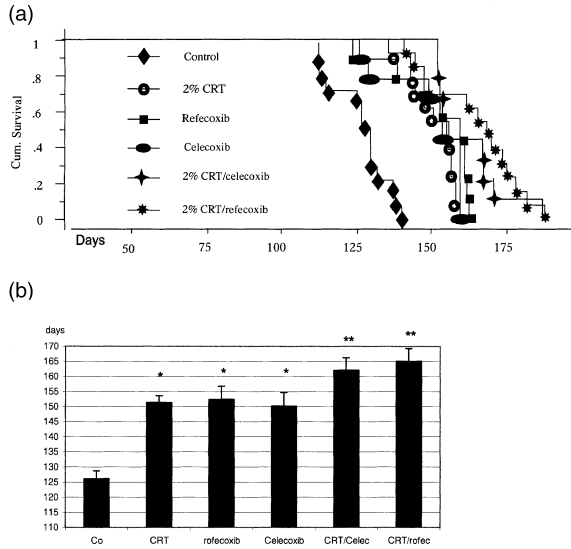


Fig. 1 Effects of 2% creatine, 0.005% rofecoxib and 0.012% celecoxib and their combination on survival in G93A transgenic mice. (a) Cumulative probability of survival. (b) Mean survival. Co, control (unsupplemented diet); CRT, 2% creatine diet; celecoxib, 0.012% celecoxib diet; rofecoxib, 0.005% rofecoxib diet; CRT/celecoxib, 2% creatine and 0.012% celecoxib diet; CRT/rofec, 2% creatine and 0.005% rofecoxib diet. * $p < 0.001$ compared with controls; ** $p < 0.05$ compared with CRT-, celecoxib- and rofecoxib-treated mice.

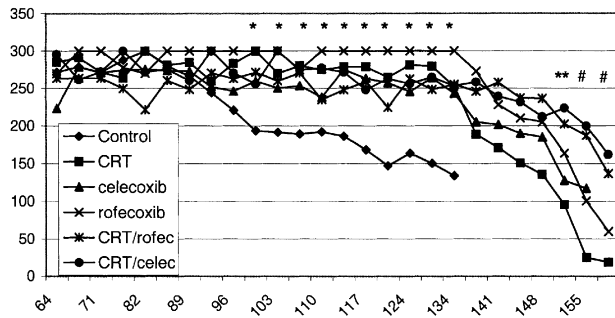


Fig. 2 Effects of 2% creatine, 0.005% rofecoxib and 0.012% celecoxib and their combination on rotarod performance in G93A transgenic mice. CRT, 2% creatine diet; celecoxib, 0.012% celecoxib; rofecoxib, 0.005% rofecoxib diet; CRT/celecoxib, 2% creatine and 0.012% celecoxib diet; CRT/rofec, 2% creatine and 0.005% rofecoxib diet. * $p < 0.05$ compared with controls; ** $p < 0.05$ compared with CRT, celecoxib; # $p < 0.05$ compared with CRT-, celecoxib- and rofecoxib-treated mice.

combinations were almost equally effective in delaying weight loss (Fig. 3).

We determined whether the observed changes in survival were associated with detectable alteration of COX-2 enzymatic activity, as assessed by PGE2 content (Fig. 4). We found that the spinal cord and cerebral cortex content of PGE2 was significantly increased in G93A mice at 110 days of age ($p < 0.001$). Treatment with COX-2 inhibitors in the

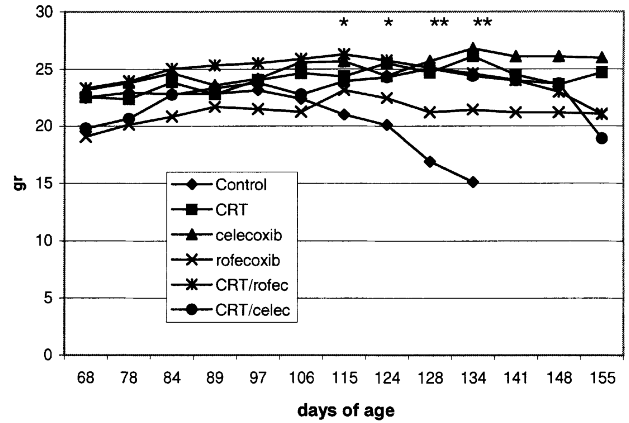


Fig. 3 Effects of 2% creatine, 0.005% rofecoxib and 0.012% celecoxib and their combination on body weight of G93A transgenic mice. Mean body weight in grams. Control, control (unsupplemented diet); CRT, 2% creatine diet; celecoxib, 0.012% celecoxib diet; rofecoxib, 0.005% rofecoxib diet; CRT/celecoxib, 2% creatine and 0.012% celecoxib diet; CRT/rofec, 2% creatine and 0.005% rofecoxib diet. * $p < 0.05$ compared with controls; ** $p < 0.01$ compared with controls.

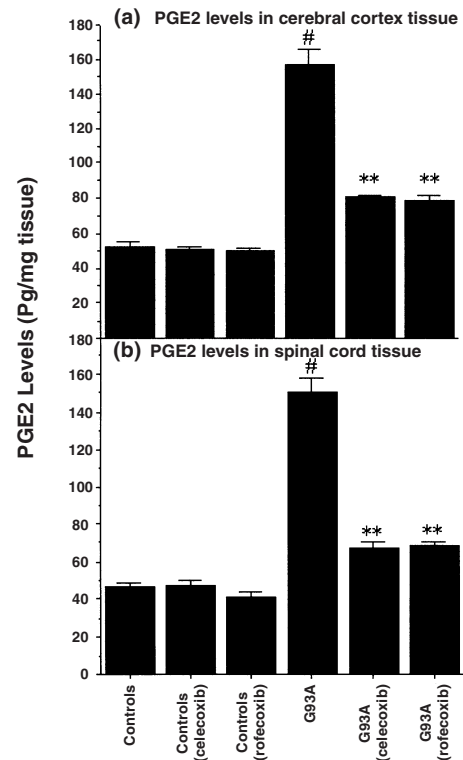


Fig. 4 Effects of 2% creatine, 0.005% rofecoxib and 0.012% celecoxib and their combination on prostaglandin E2 (PGE2) levels in (a) the cerebral cortex and (b) the spinal cord of G93A transgenic mice at 110 days of age. # $p < 0.01$ compared with control; ** $p < 0.001$ compared with untreated G93A mice. The groups studied from left to right were controls, controls (celecoxib), controls (rofecoxib), G93A, G93A (celecoxib), and G93A (rofecoxib).

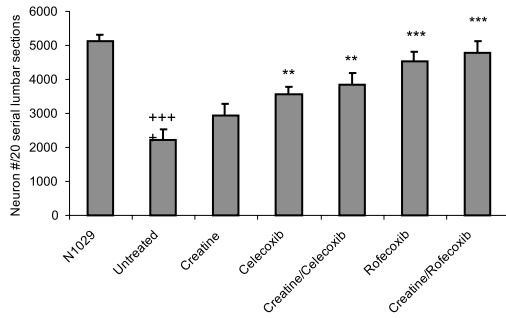


Fig. 5 Neuronal counts (mean \pm SEM) in 20 serial sections through the lumbar spinal cord from N1029 control and G93A mice with or without treatment with creatine, rofecoxib, celecoxib or their combination. *** $p < 0.001$ vs. N1029; ** $p < 0.01$ vs. untreated, *** $p < 0.001$ vs. untreated.

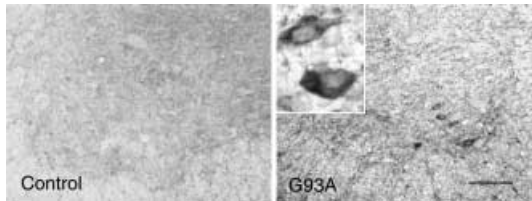


Fig. 6 Cyclooxygenase 2 immunoreactivity in the ventral horn of the lumbar spinal cord of control and G93A mice showing increased immunostaining in the G93A motor neurons. Inset is a high magnification of perikaryal staining. Scale bar, 100 μ m (20 μ m for inset).

transgenic mice significantly reduced the PGE₂ levels ($p < 0.001$). We also examined numbers of Nissl-stained neurons at 110 days of age (Fig. 5). Stereological analysis of Nissl-stained sections of the lumbar spinal cord revealed an attenuation of ventral horn neuronal loss in mice that received creatine and COX-2 inhibitors (Fig. 5). Untreated G93A mouse spinal cord sections showed a significant neuronal loss compared with N1029 controls overexpressing wild-type SOD ($p < 0.001$). Creatine supplementation increased the number of surviving motoneurons compared with unsupplemented G93A mice; however, the result was not significant, probably due to the small number of mice examined. Both rofecoxib and celecoxib provided significant neuroprotection compared with untreated transgenic mice ($p < 0.001$ and $p < 0.01$, respectively). Creatine in combination with either rofecoxib or celecoxib produced additive neuroprotective effects ($p < 0.001$ and $p < 0.01$, respectively, vs. untreated G93A mice). Lastly, we examined the localization of COX-2 in the G93A mice at 110 days of age. As shown in Fig. 6, there was a marked increase in COX-2 immunoreactivity in motoneurons in the G93A mice and, to a lesser extent, microglia which was not seen in controls.

Discussion

There is substantial evidence implicating both mitochondrial dysfunction and inflammatory mechanisms in ALS pathogenesis. Evidence for inflammation comes from studies in both transgenic mouse models and human post-mortem studies showing a robust glial reaction and microglial activation (Hirano 1996; Schiffer *et al.* 1996; Hall *et al.* 1998). The pro-inflammatory factors interleukin (IL)-6 and IL-1 β are increased in the cerebrospinal fluid and spinal cord, respectively, in ALS patients (Li *et al.* 2000; Sekizawa *et al.* 1998). Interleukin-1 β , tumor necrosis factor- α and inducible nitric oxide synthase (iNOS) levels are increased in transgenic mouse models of ALS (Ghezzi *et al.* 1998; Almer *et al.* 1999; Li *et al.* 2000; Elliott 2001). Inhibition of IL-1 β activation slows disease progression in one transgenic mouse model of ALS (Friedlander *et al.* 1998); however, in another model, an absence of IL-1 β had no effect (Nguyen *et al.* 2001).

Transcription profiling of the spinal cords of mice with the G93A SOD1 mutation showed up-regulation of tumor necrosis factor- α , CD68 and caspase 1 mRNA at 11 weeks of age prior to motor neuron death (Yoshihara *et al.* 2002). Markers of microglial activation, including CD36, MAC-1, cathepsin-S, B₂ microglobulin, C10C-alpha and AG C1Q-beta, are increased at 90 and 120 days of age (Olsen *et al.* 2001), IL- β , IL-1 β and IL-1RA are increased at 80 days and multiple caspase and death receptor components are increased at 120 days of age (Hensley *et al.* 2002).

Cyclooxygenase 2 is expressed and up-regulated in glial cells by cytokines such as IL-1 β , tumor necrosis factor- α and lipopolysaccharide (Cao *et al.* 1995; O'Banion *et al.* 1996). However, COX-2 is primarily expressed in neurons following excitotoxic lesions, apoptotic neuronal death and cerebral ischemia (Adams *et al.* 1996; Tocco *et al.* 1997; Ho *et al.* 1998; Planas *et al.* 1999; Iadecola *et al.* 2001). Cyclooxygenase 2 contributes to neuronal cell death both *in vitro* and *in vivo*. Overexpression of COX-2 can activate cell cycle genes, which may contribute to apoptotic cell death (Mirjany *et al.* 2002). Cyclooxygenase catalyzes the formation of prostaglandins, which involves reduction of the hydroperoxide resulting in the generation of free radicals. Mice overexpressing COX-2 show increased vulnerability to kainic acid and have elevated lipid peroxidation (Kelley *et al.* 1999). Neuronal death mediated by *N*-methyl-D-aspartate is diminished by COX-2 inhibitors in primary neuronal cultures (Hewett *et al.* 2000). Transgenic mice which are deficient in COX-2 show reduced susceptibility to focal ischemia and to *N*-methyl-D-aspartate neurotoxicity (Iadecola *et al.* 2001). Cyclooxygenase 2 inhibitors also protect against MPTP (Teismann and Ferger 2001).

In ALS spinal cord there is a sevenfold up-regulation of COX-2 mRNA and a 3.8-fold increase in protein levels (Yasojima *et al.* 2001). In the spinal cord of the G93A

SOD1 transgenic ALS mice there is a marked up-regulation of COX-2 mRNA in early symptomatic and end-stage illness (Almer *et al.* 2001). Furthermore, immunostaining is markedly increased in neurons and, to a lesser extent, in glia of the anterior horn of the spinal cord. In the present study, we confirmed this finding which suggests that neuronal COX-2 may play a role in the cell death observed in these mice. Measurements of COX-2 protein by western blot and catalytic activity as assessed by PGE2 content show increases which are most marked at end-stage illness in the G93A SOD1 transgenic mice (Almer *et al.* 2001). We also observed a significant increase in PGE2 levels in both the cerebral cortex and spinal cord of G93A SOD1 mice. There is a significant increase in PGE2 levels in post-mortem spinal cord and CSF of sporadic ALS patients (Almer *et al.* 2001, 2002). Cyclooxygenase 2 inhibitors block the production of PGE2 which inhibits glutamate reuptake and could exacerbate ALS in which excitotoxic mechanisms have been implicated (Bezzi *et al.* 1998). Cyclooxygenase 2 inhibitors also inhibit lipopolysaccharide-induced increases in tumor necrosis factor- α which can stimulate astrocytic glutamate release (Araki *et al.* 2001; Bezzi *et al.* 2001). Inhibition of COX-2 protects motor neurons in an organotypic model of ALS (Drachman and Rothstein 2000) and administration of the COX-2 inhibitor celecoxib increased survival in G93A SOD1 mice (Drachman *et al.* 2002).

Mitochondrial dysfunction also occurs in ALS. Mutant SOD1 is localized to the intermembrane space of mitochondria (Higgins *et al.* 2002). Mitochondrial vacuolization and swelling are amongst the earliest pathological features in two strains of transgenic ALS mice with SOD1 mutations (Gurney 1994; Wong *et al.* 1995). The mitochondrial vacuolization correlates well with a rapid deterioration in motor performance (Kong and Xu 1998) and is accompanied by reductions in the activities of electron transport enzymes and by ATP depletion which may contribute to cell death (Browne *et al.* 2001). Creatine can increase brain phosphocreatine concentrations and protect against mitochondrial dysfunction (Hemmer and Walliman 1993). Phosphocreatine serves as a direct energy source for reuptake of glutamate into synaptic vesicles and impaired glutamate uptake has been implicated in the pathogenesis of sporadic ALS (Xu *et al.* 1996; Andreassen *et al.* 2001; Wendt *et al.* 2002). We previously showed that creatine administration produces dose-dependent improvements in survival, motor performance and loss of spinal cord motor neurons in G93 SOD1 mice with a maximal effect of about 18% with 2% creatine in the diet (Klivenyi *et al.* 1999).

Therefore, in the present study we examined whether administration of the COX-2 inhibitors celecoxib or rofecoxib either alone or in combination with creatine could exert neuroprotective effects in the G93A SOD1 transgenic mouse model of ALS. We found that both celecoxib and rofecoxib

exert significant neuroprotective effects when administered alone. The effects of the two COX-2 inhibitors were similar, resulting in increases in survival of 20.6 and 19.8%, respectively. These results are consistent with a recent report by Drachman *et al.* (2002). Both agents significantly prolonged survival, delayed weight loss and improved motor performance. Furthermore, they significantly protected against motor neuron loss in the anterior horn of the lumbar spinal cord. Consistent with a previous report PGE2 levels were significantly increased in the G93A ALS transgenic mice at 110 days of age and treatment with COX-2 inhibitors significantly reduced these increases (Almer *et al.* 2001). The finding of an increase in brain PGE2 levels is consistent with recent reports of the vulnerability of cranial motor neurons as well as loss of substantia nigra neurons in G93A SOD transgenic mice (Kostic *et al.* 1997; Klivenyi *et al.* 1999; Haenggeli and Kato 2002). Another COX-2 inhibitor, nimesulide, also inhibited increases in PGE2 in G93A SOD1 transgenic mice (Pompl *et al.* 2003) Creatine alone produced a significant increase in survival of 19.8%, consistent with our previous study.

When creatine and COX-2 inhibitors were administered together they produced additive neuroprotective effects. Creatine with celecoxib improved survival by 28.6% and creatine with rofecoxib improved survival by 30.9%. This is the best improvement in survival thus far achieved by a pharmacological treatment. Furthermore, the two agents together produced additive effects on motor performance. They also showed better neuroprotection against cell loss of the anterior horn motor neurons in the lumbar spinal cord at 110 days of age. A caveat about creatine, however, is that a recent clinical trial in ALS patients failed to show a benefit (Groeneveld *et al.* 2003). This raises the possibility that either dosing is critical or that findings in this transgenic mouse model of familial ALS may not be predictive of therapeutic effects in sporadic ALS. This is less likely to be the case with COX-2 inhibitors as PGE2 levels are increased in sporadic ALS patients (Almer *et al.* 2001, 2002; Ilzecka 2003).

The results, therefore, provide further evidence that coadministration of therapeutic agents targeting different disease mechanisms may be beneficial in the treatment of ALS. It was recently shown that coadministration of minocycline with creatine produces additive neuroprotective effects with a maximal improvement in survival of 25% (Zhang *et al.* 2003). Furthermore, another recent study showed additive neuroprotective effects of riluzole, minocycline and nimodipine (Kriz *et al.* 2003). The finding that coadministration of two therapeutic agents targeting different disease mechanisms can exert additive protective effects is similar to our recent observations in transgenic mouse models of Huntington's disease (Ferrante *et al.* 2002). Both creatine and COX-2 inhibitors are safe and well tolerated in man, and therefore can be readily examined in clinical trials

(Fitzgerald and Patrono 2001) These results, therefore, suggest that administration of combinations of therapeutic agents targeting different disease mechanisms may be a useful strategy for the treatment of ALS.

Acknowledgements

The secretarial assistance of Sharon Melanson and Greta Strong is gratefully acknowledged. This work was supported by NIA grant AG 12992, the Muscular Dystrophy Association and the ALS Association.

References

- Adams J., Collaco-Moraes Y. and de Belleruche J. (1996) Cyclooxygenase-2 induction in cerebral cortex: an intracellular response to synaptic excitation. *J. Neurochem.* **66**, 6–13.
- Almer G., Vukosavic S., Romero N. and Przedborski S. (1999) Inducible nitric oxide synthase up-regulation in a transgenic mouse model of familial amyotrophic lateral sclerosis. *J. Neurochem.* **72**, 2415–2425.
- Almer G., Guegan C., Teismann P., Naini A., Rosoklija G., Hays A. P., Chen C. and Przedborski S. (2001) Increased expression of the pro-inflammatory enzyme cyclooxygenase-2 in amyotrophic lateral sclerosis. *Ann. Neurol.* **49**, 176–185.
- Almer G., Teismann P., Stevic Z., Halaschek-Wiener J., Deecke L., Kostic V. and Przedborski S. (2002) Increased levels of the pro-inflammatory prostaglandin PGE₂ in CSF from amyotrophic lateral sclerosis patients. *Neurology* **56**, A463.
- Andreassen O. A., Jenkins B. G., Dedeoglu A., Ferrante K. L., Bogdanov M. B., Kaddurah-Daouk R. and Beal M. F. (2001) Increases in cortical glutamate concentration in transgenic amyotrophic lateral sclerosis mice are attenuated by creatine supplementation. *J. Neurochem.* **77**, 383–390.
- Araki E., Forster C., Dubinsky J. M., Ross M. E. and Iadecola C. (2001) Cyclooxygenase-2 inhibitor ns-398 protects neuronal cultures from lipopolysaccharide-induced neurotoxicity. *Stroke* **32**, 2370–2375.
- Bezzi P., Carmignoto G., Pasti L., Vesce S., Rossi D., Rizzini B. L., Pozzan T. and Volterra A. (1998) Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. *Nature* **391**, 281–285.
- Bezzi P., Domercq M., Brambilla L. et al. (2001) CXCR4-activated astrocyte glutamate release via TNF α : amplification by microglia triggers neurotoxicity. *Nat. Neurosci.* **4**, 702–710.
- Browne S. E., Yang L., Fuller S. W. et al. (2001) Metabolic changes precede pathologic changes in the G93A mouse model of familial amyotrophic lateral sclerosis (ALS). *Soc. Neurosci.* **27**, 1512.
- Cao C., Matsumura K., Yamagata K. and Watanabe Y. (1995) Induction by lipopolysaccharide of cyclooxygenase-2 mRNA in rat brain; its possible role in the febrile response. *Brain Res.* **697**, 187–196.
- Drachman D. B. and Rothstein J. D. (2000) Inhibition of cyclooxygenase-2 protects motor neurons in an organotypic model of amyotrophic lateral sclerosis. *Ann. Neurol.* **48**, 792–795.
- Drachman D. B., Frank K., Dykes-Hoberg M., Teismann P., Almer G., Przedborski S. and Rothstein J. D. (2002) Cyclooxygenase 2 inhibition protects motor neurons and prolongs survival in a transgenic mouse model of ALS. *Ann. Neurol.* **52**, 771–778.
- Elliott J. L. (2001) Cytokine upregulation in a murine model of familial amyotrophic lateral sclerosis. *Brain Res. Mol. Brain Res.* **95**, 172–178.
- Ferrante R. J., Andreassen O. A., Dedeoglu A., Ferrante K. L., Jenkins B. G., Hersch S. M. and Beal M. F. (2002) Therapeutic effects of coenzyme Q10 and remacemide in transgenic mouse models of Huntington's disease. *J. Neurosci.* **22**, 1592–1599.
- Fitzgerald G. A. and Patrono C. (2001) The coxibs, selective inhibitors of cyclooxygenase-2. *N Engl. J. Med.* **345**, 433–442.
- Friedlander R. M., Brown R. H., Gagliardini V., Wang J. and Yuan J. (1998) Inhibition of ICE slows ALS in mice. *Nature* **392**, 560.
- Ghezzi P., Bernardini R., Giuffrida R., Bellomo M., Manzoni C., Comoletti D., Di Santo E., Benigni F. and Mennini T. (1998) Tumor necrosis factor is increased in the spinal cord of an animal model of motor neuron degeneration. *Eur. Cytokine Netw.* **9**, 139–144.
- Groeneveld J., Veldink J. H., van der Tweel I., Kalmijn S., Beijer C., de Visser M., Wokke J. H., Franssen H. and van den Berg L. H. (2003) A randomized trial of creatine in amyotrophic lateral sclerosis. *Ann. Neurol.* **53**, 437–445.
- Gurney M. E. (1994) Transgenic-mouse model of amyotrophic lateral sclerosis. *N. Engl. J. Med.* **331**, 1721–1722.
- Gurney M. E., Fleck T. J., Himes C. S. and Hall E. D. (1998) Riluzole preserves motor function in a transgenic model of familial amyotrophic lateral sclerosis. *Neurology* **50**, 62–66.
- Haenggli C. and Kato C. (2002) Differential vulnerability of cranial motoneurons in mouse models with motor neuron degeneration. *Neurosci. Lett.* **2002**, 39–43.
- Hall E. D., Oostveen J. A. and Gurney M. E. (1998) Relationship of microglial and astrocytic activation to disease onset and progression in a transgenic model of familial ALS. *Glia* **23**, 249–256.
- Hemmer W. and Wallimann T. (1993) Functional aspects of creatine kinase in brain. *Dev. Neurosci.* **15**, 249–260.
- Hensley K., Floyd R. A., Gordon B., Mou S., Pye Q. N., Stewart C., West M. and Williamson K. (2002) Temporal patterns of cytokine and apoptosis-related gene expression in spinal cords of the G93-SOD1 mouse model of amyotrophic lateral sclerosis. *J. Neurochem.* **82**, 365–374.
- Hewett S. J., Uliasz T. F., Vidwans A. S. and Hewett J. A. (2000) Cyclooxygenase-2 contributes to N-methyl-D-aspartate-mediated neuronal cell death in primary cortical cell culture. *J. Pharmacol. Exp. Ther.* **293**, 417–425.
- Higgins C. M., Jung C., Ding H. and Xu Z. (2002) Mutant Cu, Zn superoxide dismutase that causes motoneuron degeneration is present in mitochondria in the CNS. *J. Neurosci.* **22**, RC215.
- Hirano A. (1996) Neuropathology of ALS: an overview. *Neurology* **47**, S63–S66.
- Ho L., Osaka H., Aisen P. S. and Pasinetti G. M. (1998) Induction of cyclooxygenase (COX)-2 but not COX-1 gene expression in apoptotic cell death. *J. Neuroimmunol.* **89**, 142–149.
- Iadecola C., Niwa K., Nogawa S., Zhao X., Nagayama M., Araki E., Morham S. and Ross M. E. (2001) Reduced susceptibility to ischemic brain injury and N-methyl-D-aspartate-mediated neurotoxicity in cyclooxygenase-2-deficient mice. *Proc. Natl Acad. Sci. USA* **98**, 1294–1299.
- Ilzecka J. (2003) Prostaglandin E2 is increased in amyotrophic lateral sclerosis patients. *Acta Neurol. Scand.* **108**, 125–129.
- Kelley K. A., Ho L., Winger D., Freire-Moar J., Borelli C. B., Aisen P. S. and Pasinetti G. M. (1999) Potentiation of excitotoxicity in transgenic mice overexpressing neuronal cyclooxygenase-2. *Am. J. Pathol.* **155**, 995–1004.
- Klivenyi P., Ferrante R. J., Matthews R. T., Bogdanov M. B., Klein A. M., Andreassen O. A., Mueller G., Wermer M., Kaddurah-Daouk R. and Beal M. F. (1999) Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis. *Nat. Med.* **5**, 347–350.
- Kong J. and Xu Z. (1998) Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral sclerosis in mice expressing a mutant SOD1. *J. Neurosci.* **18**, 3241–3250.

- Kostic V., Gurney M. E., Deng H. X., Siddique T., Epstein C. J. and Przedborski S. (1997) Midbrain dopaminergic neuronal degeneration in a transgenic mouse model of familial amyotrophic lateral sclerosis. *Ann. Neurol.* **41**, 497–504.
- Kriz J., Gowing C. and Julien J. P. (2003) Efficient three-drug cocktail for disease induced by mutant superoxide dismutase. *Ann. Neurol.* **53**, 429–436.
- Li M., Ona V. O., Guegan C. *et al.* (2000) Functional role of caspase-1 and caspase-3 in an ALS transgenic mouse model. *Science* **288**, 335–339.
- Mirjany M., Ho L. and Pasinetti G. M. (2002) Role of cyclooxygenase-2 in neuronal cell cycle activity and glutamate-mediated excitotoxicity. *J. Pharmacol. Exp. Ther.* **301**, 494–500.
- Nguyen M. D., Julien J. P. and Rivest S. (2001) Induction of proinflammatory molecules in mice with amyotrophic lateral sclerosis: no requirement for proapoptotic interleukin-1beta in neurodegeneration. *Ann. Neurol.* **50**, 630–639.
- O'Banion M. K., Miller J. C., Chang J. W., Kaplan M. D. and Coleman P. D. (1996) Interleukin-1 beta induces prostaglandin G/H synthase-2 (cyclooxygenase-2) in primary murine astrocyte cultures. *J. Neurochem.* **66**, 2532–2540.
- Olsen M. K., Roberds S. L., Ellerbrock B. R., Fleck T. J., McKinley D. K. and Gurney M. E. (2001) Disease mechanisms revealed by transcription profiling in SOD1-G93A transgenic mouse spinal cord. *Ann. Neurol.* **50**, 730–740.
- Planas A. M., Soriano M. A., Justicia C. and Rodriguez-Farre E. (1999) Induction of cyclooxygenase-2 in the rat brain after a mild episode of focal ischemia without tissue inflammation or neural cell damage. *Neurosci. Lett.* **275**, 141–144.
- Pompl P. N., Ho L., Bianchi M., McManus T., Qin W. and Pasinetti G. M. (2003) A therapeutic role for cyclooxygenase-2 inhibitors in a transgenic mouse model of amyotrophic lateral sclerosis. *FASEB J.* February 5 (epub ahead of print).
- Rosen D. R., Siddique T., Patterson D. *et al.* (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* **362**, 59–62.
- Schiffer D., Cordera S., Cavalla P. and Migheli A. (1996) Reactive astrogliosis of the spinal cord in amyotrophic lateral sclerosis. *J. Neurol. Sci.* **139**, 27–33.
- Sekizawa T., Openshaw H., Ohbo K., Sugamura K., Itoyama Y. and Niland J. C. (1998) Cerebrospinal fluid interleukin 6 in amyotrophic lateral sclerosis: immunological parameter and comparison with inflammatory and non-inflammatory central nervous system diseases. *J. Neurol. Sci.* **154**, 194–199.
- Teismann P. and Ferger B. (2001) Inhibition of the cyclooxygenase isoenzymes COX-1 and COX-2 provide neuroprotection in the MPTP-mouse model of Parkinson's disease. *Synapse* **39**, 167–174.
- Tocco G., Freire-Moar J., Schreiber S. S., Sakhi S. H., Aisen P. S. and Pasinetti G. M. (1997) Maturational regulation and regional induction of cyclooxygenase-2 in rat brain: implications for Alzheimer's disease. *Exp. Neurol.* **144**, 339–349.
- Wendt S., Dedeoglu A., Speer O., Wallimann T., Beal M. F. and Andreassen O. A. (2002) Reduced creatine kinase activity in transgenic amyotrophic lateral sclerosis mice. *Free Radic. Biol. Med.* **32**, 920–926.
- Wong P. C., Pardo C. A., Borchelt D. R., Lee M. K., Copeland N. G., Jenkins N. A., Sisodia S. S., Cleveland D. W. and Price D. L. (1995) An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. *Neuron* **14**, 1105–1116.
- Xu C. J., Klunk W. E., Kanfer J. N., Xiong Q., Miller G. and Pettegrew J. W. (1996) Phosphocreatine-dependent glutamate uptake by synaptic vesicles. *J. Biol. Chem.* **271**, 13 435–13 440.
- Yasojima K., Tourtellotte W. W., McGeer E. G. and McGeer P. L. (2001) Marked increase in cyclooxygenase-2 in ALS spinal cord: implications for therapy. *Neurology* **57**, 952–956.
- Yoshihara T., Ishigaki S., Yamamoto M., Liang Y., Niwa J., Takeuchi H., Doyu M. and Sobue G. (2002) Differential expression of inflammation- and apoptosis-related genes in spinal cords of a mutant SOD1 transgenic mouse model of familial amyotrophic lateral sclerosis. *J. Neurochem.* **80**, 158–167.
- Zhang W., Narayanan M. and Friedlander R. M. (2003) Additive neuroprotective effects of minocycline with creatine in a mouse model of ALS. *Ann. Neurol.* **53**, 267–270.