The Antioxidant *N*-Acetylcysteine Does Not Delay Disease Onset and Death in a Transgenic Mouse Model of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder of motoneurons causing progressive paralysis, inevitably leading to death. The identification of point mutations in the cytosolic copper/zinc superoxide dismutase (SOD1) gene in a subset of ALS patients has led to the hypothesis that, at least in a subset of ALS patients, free radical damage contributes to motoneuron death.[1] *N*-Acetylcysteine (NAC) can act as an antioxidant, either directly as a free radical scavenger or as a precursor of glutathione and metallothioneine, and may act as a chelator, which facilitates disposal of toxic metals. A mild positive, albeit nonsignificant, effect of NAC on survival has been observed in a clinical trial with ALS patients,[2] and on motoneuron degeneration in wobbler mice (an animal model for juvenile spinomuscular atrophy).[3] NAC is clinically safe and has been shown to pass the blood–brain barrier.[2] To examine whether NAC may benefit ALS patients with SOD1 mutations, we have tested NAC in a transgenic mouse model of ALS expressing human SOD1 with a Gly^{93} to Ala (G93A) mutation.

Transgenic G93A mice (Jackson Laboratories, Bar Harbor, ME) were descended of the Gurney G1L line[4] but showed a slower clinical and pathological progression of disease, probably due to a 30% reduction in transgene copy number. Sex- and weight-matched G93A mice were randomly selected across litters. They were housed individually with a 12-h reversed dark/light cycle and were fed ad libitum with a standard diet (AM2-14-10, Hope Farm, Woerden, The Netherlands). NAC treatment was started at 120 days of age, before onset of paresis.[5] Onset and progression of clinical disease was scored by examining the mice weekly for extension of the limbs when suspended in the air by their tail and for grip strength. Mice were killed (= end-stage disease) when they could not right themselves or when they developed a severe eye infection. NAC solution (50 mg/ml; see Louwerse and colleagues[2]) was injected subcutaneously at a dose of 0.5 mg/g of body weight daily at the end of the dark period. This treatment protocol resulted in NAC plasma concentrations of 31 ± 9 (mean ± SD) μg/ml at 1 hour after injection, elimination half-life being approximately 50 minutes. NAC-treated mice did not show obvious behavioral abnormalities but showed a 4 to 8% weight reduction ($p < 0.01$, unpaired Student's t test), compared with control groups, ie, untreated and G93A mice injected daily with saline. NAC injections did not significantly (unpaired Student's t tests) influence age of clinical onset and survival of G93A mice compared with control groups (Table). In a second experiment, NAC (10 mg/ml)[3] was supplemented to the drinking water, resulting in plasma concentrations of 0.5 to 2 μg/ml. As in the injected animals, NAC treatment resulted in a small weight loss but had no significant effect on disease onset and survival (see Table).

In sum, our results indicate that treatment with the antioxidant NAC does not influence disease onset and survival in transgenic mice carrying an ALS-linked SOD1 mutation. In a similar manner, Gurney and collaborators[4] showed that dietary supplementation with high doses of vitamin E did not improve survival in SOD1-ALS transgenic mice. These data suggest that antioxidant treatment will not have a beneficial effect on ALS patients with SOD1 mutations.

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References

1. Siddique T. ALS: molecular clues to the jigsaw puzzle of neuronal degeneration. Cold Spring Harb Symp Quant Biol 1996;61: 699–708
2. Louwerse ES, Weverling GJ, Bossuyt PM, et al. Randomized, double-blind, controlled trial of acetylcysteine in amyotrophic lateral sclerosis. Arch Neurol 1995;52:559–564
3. Henderson JT, Javaheri M, Kopko S, Roder JC. Reduction of lower motor neuron degeneration in wobbler mice by *N*-acetyl-L-cysteine. J Neurosci 1996;16:7574–7582
4. Gurney ME, Cuttings FB, Zhai P, et al. Benefit of vitamin E, riluzole, and gabapentin in a transgenic model of familial amyotrophic lateral sclerosis. Ann Neurol 1996;39:147–157
5. Jaarsma D, Holstege JC, Troost D, et al. Induction of c-Jun immunoreactivity in spinal cord and brainstem neurons in a transgenic mouse model for amyotrophic lateral sclerosis. Neurosci Lett 1996;219:179–182

Table. Effect of N*-Acetylcysteine Treatment on Disease Onset and Survival of G93A Transgenic Mice*

		Age at Onset (days)					Age at Death (days)				
Treatment (n)	Control (n)	Treatment Group (Mean ± SEM)	Control Group (Mean ± SEM)	Δ	t	p	Treatment Group (Mean ± SEM)	Control Group (Mean ± SEM)	Δ	t	p
NAC injections (14)	No treatment (14)	205 ± 7	205 ± 7	0	0.029	NS	251 ± 7	250 ± 9[a]	1	0.896	NS
	Saline injections (15)		195 ± 3	10	1.298	NS		239 ± 5[a]	12	1.433	NS
NAC drinking water (7)	No treatment (8)	189 ± 4	196 ± 7	−7	0.773	NS	233 ± 4	237 ± 8	−4	0.484	NS

[a]There is no significant statistical difference between the two control groups (unpaired Student's t test).

NAC = *N*-Acetylcysteine.