Free Radical Research, March 2008; 42(3): 221–225

informa
healthcare

Increased mitochondrial oxidative damage and oxidative DNA damage contributes to the neurodegenerative process in sporadic amyotrophic lateral sclerosis

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Accepted by Professor H. Poulsen

(Received 10 October 2007; revised 30 November 2007)

Abstract
To investigate the possibility that mitochondrial oxidative damage or oxidative DNA damage or both contribute to the neurodegenerative process of sporadic amyotrophic lateral sclerosis (sALS), this study used high-performance liquid chromatography with an electrochemical detector to measure the concentrations of the reduced and oxidized forms of coenzyme Q10 (CoQ10) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) in the cerebrospinal fluid (CSF) of 17 patients with sALS and 17 age-matched controls with no neurological diseases. The percentage of oxidized CoQ10 in the CSF of sALS patients was greater than that in the CSF of controls ($p < 0.002$) and was negatively correlated with the duration of illness ($\rho = -0.61$, $p < 0.01$). The concentration of 8-OHdG in the CSF of sALS patients was greater than that in the CSF of controls ($p < 0.005$) and was positively correlated with the duration of illness ($\rho = 0.53$, $p < 0.005$). The percentage of oxidized CoQ10 was correlated with the concentrations of 8-OHdG in the CSF of sALS patients ($\rho = -0.53$, $p < 0.05$). These results suggest that both mitochondrial oxidative damage and oxidative DNA damage play important roles in the pathogenesis of sporadic amyotrophic lateral sclerosis.

Keywords: *Sporadic amyotrophic lateral sclerosis, coenzyme Q10, 8-hydroxy-2′-deoxyguanosine, cerebrospinal fluid*

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder caused by the degeneration and eventual death of motor neurons in the brainstem, spinal cord, and motor cortex. The disease was first described by Charcot in 1869, but its cause remains unknown and there is still no way to prevent or cure it. In 1993 attention was drawn to the possible participation of a free radical in the aetiology of ALS by the discovery that point mutations in the gene that produces Cu/Zn-binding superoxide dismutase (SOD) are associated with the familial ALS inherited as an autosomal dominant trait [1,2,3]. Several recent studies have also established that oxidative stress is associated with ALS. They found familial and sporadic ASL (sALS) patients to have increased levels of nuclear 8-hydroxy-2′-deoxyguanosine (8-OHdG) in the motor cortex [4] and lower glutathione peroxidase activity in the precentral gyrus [5]. Plasma levels of 8-OHdG are higher in sALS patients than in healthy controls [6], but plasma and serum levels of the antioxidants vitamin E [7–9], ascorbic acid [10], and coenzyme Q10 (CoQ10) [9,11] do not differ between sALS patients and healthy controls. Moreover, the levels of nitrate and 3-nitrotyrosine in the cerebrospinal fluid (CSF) of sALS patients are significantly higher than those in

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ISSN 1071-5762 print/ISSN 1029-2470 online
DOI: 10.1080/10715760701877262

the CSF of healthy controls [12], whereas the levels of α-tocopherol quinine are remarkably lower than those in the CSF of healthy controls [13].

There has been recent interest in the role that mitochondria might play in the pathogenesis of sALS. Coenzyme Q10 (CoQ10)—associated with the activity of mitochondria complexes I, II and III [14]—is an enzyme cofactor in the mitochondrial electron transport chain and thus plays an important role in cellular respiration. It also promotes bio-oxidation reactions and in its reduced form is an antioxidant protecting cells from the destructive effects of free radicals. Because vitamin C and the reduced form of CoQ10 are among the first antioxidants lost when plasma is under oxidative stress, the redox status of CoQ10 (the ratio of the oxidized form to the total CoQ10) should be an early marker of oxidative stress [13].

Superoxide dismutase catalyses the conversion of reactive oxygen to oxygen and hydrogen peroxide, which is usually decomposed to oxygen and water by the enzymes catalase and glutathione peroxidase. In the presence of ferrous iron, however, hydrogen peroxide generates hydroxyl radicals by the Fenton reaction and these extremely reactive free radicals generate 8-OHdG by oxidizing the guanine of DNA. The concentrations of 8-OHdG in plasma and CSF also increased with age, providing evidence for a role of oxidative damage in normal ageing. The plasma, urine and cerebrospinal fluid concentrations of 8-OHdG in sALS patients are significantly higher than those in controls [5].

In the present study, investigating the possibility that mitochondrial oxidative damage or oxidative DNA damage or both contribute to the neurodegenerative process in the patient of sporadic amyotrophic lateral sclerosis, we measured the concentrations of the reduced and oxidized forms of CoQ10 and 8-OHdG, DNA oxidative stress markers, in the CSF of patients with sALS.

Subjects and methods

Subjects

The subjects were 17 untreated patients with sALS (aged 63.7 ± 12.2 years [mean $\pm$ SD]) and 17 controls (63.8 ± 16.4 years). The controls were neurologically normal patients who underwent lumbar anaesthesia for minor surgery. The clinical diagnosis of sALS was based on the WFN diagnostic criteria and the severity of the disease was evaluated using the ALS index developed by Jableki et al. [15], which is derived by adding disability scores in 12 areas to obtain an ALS score between 0–40. The patients had ALS scores ranging from 2.0–23.0 (12.3 ± 6.8) and their illness durations ranged from 0.3–3.0 (1.1 ± 0.7) years. All patients were admitted to a hospital and maintained on a standard diet and informed consent was obtained from the patients or their families.

CSF analysis

Four-millilitre samples of CSF were obtained, by lumbar puncture with the subjects in a lateral decubitus position, between 9:00 and 10:00 am after overnight bed-rest and before breakfast. The first 3 ml was used for general examination and the next 1 ml was rapidly frozen and stored at -80°C until its concentrations of CoQ10 and 8-OHdG were measured.

The concentrations of the reduced and oxidized forms of CoQ10 were measured using a modified version of the method used by Yamashita and Yamamoto [16]. Briefly, 200 μl of CSF was combined with 200 μl of ethanol, mixed with 1 ml of n-hexane and centrifuged. An 800-μl sample was removed from the top hexane layer, dried under a stream of nitrogen and reconstituted with ethanol. The ethanol solution was analysed using a high-performance liquid chromatography column (MCM C18 reversed-phase column, 250×4.6 mm; MC Medical, Tokyo, Japan) with an electrochemical detector (Coulochem II Model 5200; ESA Inc., Bedford, MA). The mobile phase consisted of 50 mm sodium perchlorate in methanol. The electrode potentials were maintained at 0.4 V for the guard cell, 0.4 V for electrode I and 0.3 V for electrode II. The flow rate was 1.0 ml/min and the column temperature was kept at 25°C. The detection limit for CoQ10 was 0.2 nm.

The concentration of 8-OHdG was determined using a modified version of the method used by Shigenaga et al. [17]. Briefly, 1 ml of CSF was absorbed in a solid-phase extraction cartridge (Bond Elut C18, Varian Inc., Harbor City, CA) and eluted with 1.5 ml of methanol. The eluent was concentrated with a centrifugal evaporator and dissolved in 100 μl of distilled water. After filtration with a 0.45-μm membrane filter, 40 μl of the solution was analysed using high-performance liquid chromatography (MCM C18 reversed-phase column, 250×4.6 mm; MC Medical, Tokyo, Japan) with an electrochemical detector (Coulochem II Model 5200; ESA Inc., Bedford, MA). The mobile phase consisted of 10 mm NaH2PO4 and 6% methanol. The electrode potentials were maintained at 0.3 V for the guard cell, 0.15 V for electrode I and 0.25 V for electrode II. The flow rate was 1.0 ml/min and the column temperature was kept at 20°C. The limit of detection for 8-OHdG was 20 pm.

Statistical analysis

The Mann-Whitney U-test and Spearman's rank correlation coefficient ρ were used for statistical analyses in which the significance level was set at $p < 0.05$.

Results

The CSF concentration of reduced CoQ10 did not differ significantly between sALS patients and controls, but the percentage of oxidized CoQ10 in the CSF of sALS patients was significantly greater than that in the CSF of controls ($85.7\% \pm 4.6\%$ vs $68.2\% \pm 20.4\%$; $p < 0.002$) (Figure 1). The percentage of oxidized CoQ10 in the CSF of sALS patients was inversely correlated with the duration of illness ($\rho = -0.61$, $p < 0.01$) but was not significantly correlated with the ALS score (Figure 2).

The CSF concentration of 8-OHdG, on the other hand, was significantly greater in sALS patients than in controls (5.1 ± 3.4 pg/ml vs 1.7 ± 1.3 pg/ml; $p < 0.005$) (Figure 3). The concentration of 8-OHdG in the CSF of sALS patients was correlated with the duration of illness ($\rho = 0.53$, $p < 0.005$) but was not significantly correlated with the ALS score (Figure 4). Furthermore, the percentage of oxidized CoQ10 in the CSF of sALS patients was inversely correlated with the concentration of 8-OHdG there ($\rho = -0.53$, $p < 0.05$).

Discussion

Mitochondria play an important role in a production of ATP and the control of apoptosis. Normal mitochondria produce ATP by oxidative phosphorylation, but when the function is lost reactive oxygen leaks from the membranes lining the mitochondria and causes cytotoxicity. When mitochondrial DNA (mtDNA) is injured by reactive oxygen, the synthesis of the enzymes in the electron transport chain is impaired, reducing the production of ATP and increasing the leakage of reactive oxygen. mtDNA is unstable because it does not have protection proteins like the histones protecting nuclear DNA and because the mechanisms repairing mitochondrial DNA are weaker than those repairing nuclear DNA [18]. In addition, mtDNA is particularly susceptible to oxidative damage because it contacts the internal membranes of mitochondria, which produce the most reactive oxygen in a cell. On the other hand, a motoneuron has a very long axon in comparison with the axon of other central neurons and requires a constant supply of energy to produce action potentials. Motoneurons are therefore thought to be easily affected by mitochondrial abnormalities.

Most of the toxic superoxide produced in the mitochondrial electron transport chain is formed in Complex III and Complex I and mitochondria contain a form of superoxide dismutase with manganese called superoxide dismutase 2 (SOD2). The SOD1 in cells was first thought to be present only in

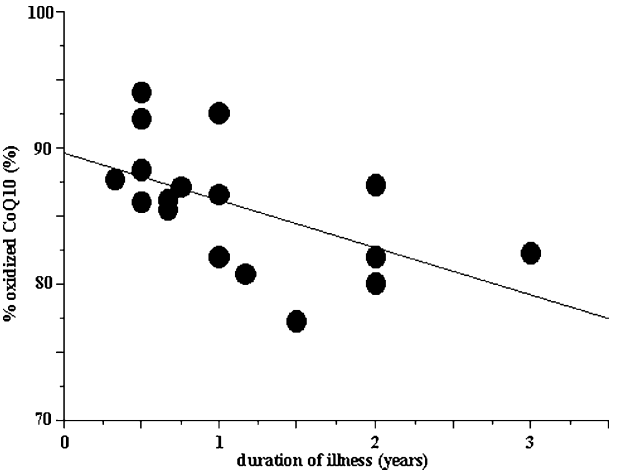

Figure 2. Percentage of the total CoQ 10 in the cerebrospinal fluid that is oxidized vs the duration of illness for patients with sporadic amyotrophic lateral sclerosis.

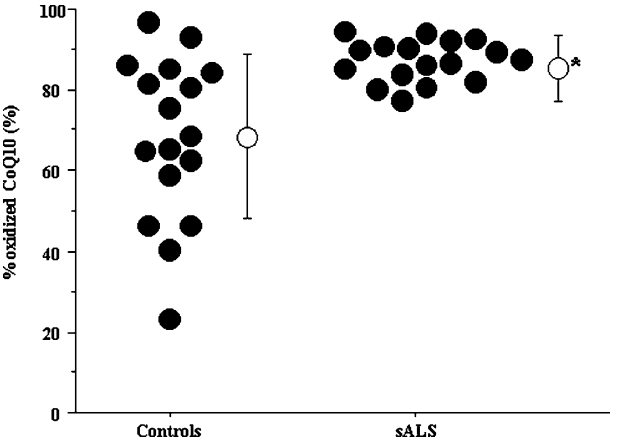

Figure 1. Percentage of the total CoQ 10 in the cerebrospinal fluid that is oxidized in controls and in patients with sporadic amyotrophic lateral sclerosis (sALS). Open circles with bars show mean ± SEM. * $p < 0.002$ compared with controls by using the Mann-Whitney U-test.

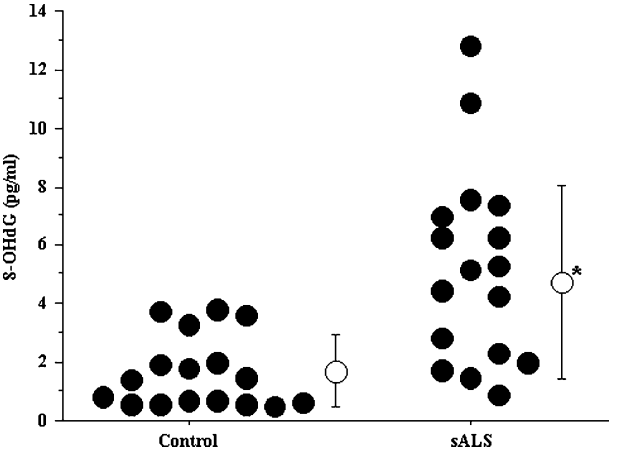

Figure 3. The concentration of 8-OHdG in the cerebrospinal fluid that is oxidized in controls and in patients with sporadic amyotrophic lateral sclerosis (sALS). Open circles with bars show mean ± SEM. * $p < 0.005$ compared with controls by using the Mann-Whitney U-test.

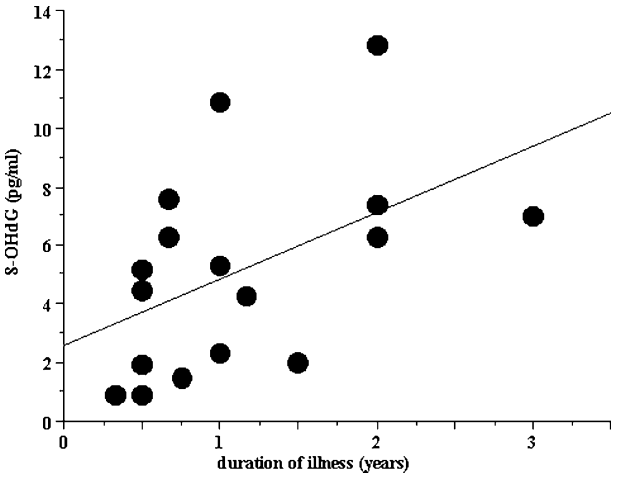

Figure 4. The concentration of 8-OHdG in the cerebrospinal fluid vs the duration of illness for patients with sporadic amyotrophic lateral sclerosis.

the cytoplasm, but 1–5% of it has recently been found to be between the internal and external membranes of mitochondria and to protect mitochondria from oxidative stress [19,20]. Mitochondria with abnormally swollen christae and torn external membranes are found in motoneurons before the clinical manifestations of ALS appear [21–23]. Furthermore, a mutant SOD1 has been found in the degenerated mitochondria of transgenic mouse and it is supposed that mutated SOD1 contributes to mitochondrial abnormality in ALS [24]. It has been reported that the progression of ALS can be slowed by massive doses of CoQ10 [25], so the relation between ALS and mitochondria dysfunction is important. While the total CoQ10 in the serum of sALS patients has been reported to differ insignificantly from that in the serum of controls [10], the percentage of oxidized CoQ10 in the serum of sALS patients has been reported to be significantly greater than in the serum of controls [26]. Thus, a consensus has not been obtained.

In this study, the percentage of oxidized CoQ10 in the CSF of sALS patients was significantly greater than that in the CSF of the controls. The percentage of oxidized CoQ10 in the CSF of sALS patients was not significantly correlated with the ALS score, but the percentage of oxidized CoQ10 in the CSF of sALS patients was significantly inversely correlated with the duration of illness. These findings suggest that an oxidation disorder of mitochondria occurred in the early stage of sALS and they are consistent with the conventional view that mitochondrial oxidative damage might contribute to the onset of sALS.

Since 8-OHdG is generated by oxidizing the guanine of DNA, 8-OHdG is regarded as one of the best indexes of DNA disorder due to oxidative stress. 8-OHdG is found in the anterior horn neurons of G93A SOD1 transgenic mice when they are 25 weeks old, before these neurons are lost and ALS-like symptoms appear, and it progressively accumulates until at 35 weeks symptoms appear and it is found in both the anterior and posterior horns [27]. Since 8-OHdG is not found in the anterior horn neurons of wild-type mice, the mitochondrial abnormality in a G93A SOD1 transgenic mouse seems to be an early marker of eventual onset of symptoms. Furthermore, immunohistochemical studies showed increased neuronal staining for 8-OHdG in the spinal cord of sALS patients [27] and the concentrations of 8-OHdG have been found to be increased in the motor cortex (Brodmann area 4) and spinal cord of sALS [3,28]. The plasma, urine and cerebrospinal fluid concentrations of 8-OHdG in sALS patients are significantly higher than those in controls [5]. The concentrations of 8-OHdG in plasma and CSF also increased with age, providing evidence for a role of oxidative damage in normal ageing. These findings suggest that oxidative DNA damage accompanies the neurodegenerative process in sALS.

In this study, the concentrations of 8-OHdG in the CSF of sALS patients were significantly higher than those in the CSF of the controls. The 8-OHdG concentrations in the CSF of sALS patients were not correlated with the ALS scores of those patients, but they were positively correlated with the durations of illness. These findings suggest that the oxidation of DNA and the production of 8-OHdG are enhanced in sALS. They support the conventional view that oxidative DNA damage might contribute to the onset of sALS.

These results suggested that both mitochondrial oxidative damage and oxidative DNA damage play important roles in the neurodegenerative process of sALS. Which mitochondrial oxidative damage or oxidative DNA damage is the primary event in the pathogenesis of sALS, however, is unknown. Since we do not yet know where 8-OHdG is produced in the patients with sALS, we surmise but can not yet prove that mitochondrial turnover lead to 8-OHdG release by digestion of DNA. There is no evidence that 8-OHdG in cerebrospinal fluid can come from mitochondria, although it has been suggested. Moreover, there is no known DNA repair mechanism at least in mitochondria that could give rise to such an excretion. At present, the hydroxylation of guanine, which is one of the frequent DNA base modifications following oxidative stress, is thought to be generated either by direct oxidation of the base in DNA, by hydroxyl radicals or peroxynitrite or by incorporation of the oxidized nucleoside triphosphate into DNA during synthesis or repair. However, it cannot be

determined whether accumulation of DNA is due to increased oxidative stress, to decreased repair or to decreased transport over the blood–brain barrier.

Acknowledgements

We wish to thank Miss Yoko Iwa for her technical and secretarial assistance.

References

[1] Siddique T, Figlewicz DA, Pericak-Vance MA, Haines JL, Rouleau G, Jeffers AJ, et al. Linkage of a gene causing familial amyotrophic lateral sclerosis to chromosome 21 and evidence of genetic-locus heterogeneity. N Engl J Med 1991;324:1381–1384.

[2] Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hertati A, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 1993;362:59–62.

[3] Ferrante RJ, Bowne SE, Shinobu LA, Bowling AC, Baik MJ, MacGarvey U, et al. Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis. J Neorochem 1997;69:2064–2074.

[4] Przedboski S, Donaldson DM, Jakowec M, Kish SJ, Guttman M, Rosoklija G, et al. Brain superoxide dismutase, catalase and glutathione peroxidase activities in amyotrophic lateral sclerosis. N Engl Ann Neurol 1996;39:158–165.

[5] Bogdanov M, Brown Jr RH, Matson W, Smart R, Hayden D, O'Donnell H, et al. Increased oxidative damage to DNA in ALS patients. Free Radic Biol Med 2000;29:652–658.

[6] Oteiza PI, Uchitel OD, Carrasquedo F, Dubrovski AL, Roma JC, Fraga CG. Evaluation of antioxidants, proteins and lipid oxidation products in blood from sporadic amyotrophic lateral sclerosis patients. Neorochem Res 1997;22:535–539.

[7] Bonnefont-Rousselot D, Lacomblez L, Jaudon M, Lepage S, Salachas F, Bensiman G, et al. Blood oxidative stress in amyotrophic lateral sclerosis. J Neurol Sci 2000;178:57–62.

[8] Iwasaki Y, Ikeda K, Kinoshita M. Vitamin A and E levels are normal in amyotrophic lateral sclerosis. J Neurol Sci 1995;29:652–658.

[9] Paraskevas GP, Kapaki E, Libitaki G, Zournas C, Segditsa I, Papageorgiou C. Ascorbate in healthy subjects, amyotrophic lateral sclerosis and Alzheimer's disease. Acta Nerol Scand 1997;96:88–90.

[10] Molina JA, de Bustos F, Jimenez-Jimenez FJ, Gomez-Escalonilla C, Garcia-Redonda A, Esteban J, et al. Serum levels of coenzyme Q10 in patients with amyotrophic lateral sclerosis. J Neural Transm 2000;107:1021–1026.

[11] Tohgi H, Abe T, Yamazaki K, Murata T, Ishizaki E, Isobe C. Remarkable increase in cerebrospinal fluid 3-nitrotyrosine in patients with sporadic amyotrophic lateral sclerosis. Ann Neurol 1999;46:129–131.

[12] Tohgi H, Abe T, Saheki M, Yamazaki K, Takahashi S. Tocopherol quinone level is remarkably low in the cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis. Neurosci Lett 1996;207:5–8.

[13] Yamamoto Y, Kawamura M, Tatsuno K, Yamashita S, Niki E, Naito C, et al. Formation of lipid hydroperoxides in the cupiric ion-induced oxidation of plasma and low density lipoprotein. In: Davies KJA, editor. Oxidative damage and repair. New York: Pergamon Press; 1991. p 287–291.

[14] Shults CW, Haas RH, Passov D, Beal MF. Coenzyme Q10 levels correlate with the activities of complexes I and II/III in mitochondria from parkinsonian and nonparkinsonian subjects. Ann Neurol 1997;42:261–264.

[15] Jablecki CK, Berry, Leach J. Survival prediction in amyotrophic lateral sclerosis. Muscle Nerve 1989;12:833–841.

[16] Yamashita S, Yamamoto Y. Simultaneous detection ob ubiquinol and ubiquinone in human plasma as a marker of oxidative stress. Anal Biochem 1997;250:66–73.

[17] Shigenaga MK, Gimeno CJ. Ames BN. Urinary 8-hydroxy-27′-deoxyguanosine as a biological marker of in vivo oxidative DNA damage. Proc Natl Acad Sci USA 1989;86:9697–9701.

[18] Richter C, Park JW, Ames BN. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. Proc Natl Acad Sci USA 1988;85:6465–6467.

[19] Mattiazzi M, D'Aurelio M, Gajewski CD, Martushava K, Kiaei M, Flint M, et al. Mutated human SOD1 causes dysfunction of oxidative phosphorylation in mitochondria of transgenic mice. J Biol Chem 2002;277:29626–29633.

[20] Struz LA, Diekert K, Jensen LT, Lill R, Cizewski Culotta V, et al. A fraction of yeast Cu/Zn superoxide dismutase and its metallochaperone, CCS, localize to the intermembrane space of mitochondria: a physiological role for SOD1 in guarding against mitochondrial oxidative damage. J Biol Chem 2001;276:38084–38089.

[21] Borthwick GM, Johnson MA, Ince PG, Shaw PJ, Turnbull DM. Mitochondrial enzyme activity in amyotrophic lateral sclerosis: Implications for the role of mitochondria in neuronal cell death. Ann Nerol 1999;46:787–790.

[22] Bowling AC, Schulz JB, Brown RH, Beal MF. Superoxide dismutase activity. Oxidative damage, and mitochondrial energy metabolism in familial and sporadic amyotrophic lateral sclerosis. J Neurochem 1993;61:2322–2325.

[23] Kong J, Xu Z. Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral sclerosis in mice expressing a mutant SOD1. J Neurosci 1998;18:3241–3250.

[24] Jaarsma D, Rognoni F, Duijn WV, Verspaget HW, Haasdijk ED, Holstege JC. Cu/Zn superoxide dismutase (SOD1) accumulates in vacuolated mitochondria in transgenic mice expressing amyotrophic lateral sclerosis-linked SOD1 mutations. Acta Neuropathol 2001;102:293–305.

[25] Beal MF. Coenzyme Q10 as a possible treatment for neurodegenerative disease [Review][61 refs]. Free Radic Res 2002;36:455–460.

[26] Sohmiya M, Tanaka M, Suzuki Y, Tanino Y, Okamoto K, Yamamoto Y. An increase of oxidized coenzyme Q-10 occurs in the plasma of sporadic ALS patients. J Neurol Sci 2005;228:49–53.

[27] Warita H, Hayashi T, Murakami T, Manabe Y, Abe K. Oxidative damage to mitochondrial DNA in spinal motoneurons of transgenic ALS mice. Mol Brain Res 2001;89:147–152.

[28] Fitzmaurice PS, Shaw IC, Kleiner HE, Miller RT, Monks TJ, Lau SS, et al. Evidence for DNA damage in amyotrophic lateral sclerosis. Muscle Nerve 1996;19:797–798.