Free Radical Research, April 2005; 39(4): 383–388

Increased oxidative damage to DNA in an animal model of amyotrophic lateral sclerosis

NORBERTO AGUIRRE[1], M. FLINT BEAL[2], WAYNE R. MATSON[3], & MIKHAIL B. BOGDANOV[3]

[1]*Department of Pharmacology, University of Navarra Medical School, Pamplona Medical School, Pamplona, Spain,* [2]*Department of Neurology and Neuroscience, Weill Medical College of Cornell University, New York Presbyterian Hospital, New York, NY, USA, and* [3]*ESA, Inc., Chelmsford, MA, USA*

Accepted by Professor H. E. Poulsen

(Received 2 September 2004; acccepted 25 November 2004)

Abstract

Substantial evidence suggest that oxidative damage may play a role in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS). We examined levels of 8-Hydroxy-2′-deoxyguanosine (8OH2′dG) in the nuclear DNA from the spinal cord, frontal cortex, striatum and cerebellum from G93A mice at 60, 90, and 120 days of age. We also used *in vivo* microdialysis to measure free levels of 8OH2′dG and 8-Hydroxyguanine (8OHG) at the same time points in the frontal cortex of G93A mice. Increased 8OH2′dG DNA levels were observed in the spinal cord (at 60, 90 and 120 days), in the cortex (at 90, and 120 days), and in the striatum (at 120 days), as compared to age-matched littermate controls. No significant changes were found in the cerebellum at any of the time points studied. Free levels of 8OH2′dG in the cortex of G93A mice were increased, as compared to control mice, at 90 and 120 days. Free levels of 8OHG were found to be significantly higher at 120 days of age in control mice than in G93A mice. These results provide evidence that in this model of ALS oixidative DNA-damage is increased and base excision-repair may be deficient.

Keywords: *Amyotrophic lateral sclerosis, transgenic, DNA, oxidative damage, 8-hydroxy-2′-deoxyguanosine, 8-hydroxyguanine*

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease resulting in degeneration of motor neurons in lower brain stem and spinal cord, and of cortical motor neurons, leading to generalized weakness and muscle dystrophy followed by death [1]. The majority of the cases are sporadic (SALS) with unknown causes. A total of 10–15% of the cases are inherited (familial ALS (FALS)), and of these approximately 25% are associated with the point mutations in the gene encoding cytosolic Cu/Zn superoxide dismutase (SOD1) [2]. Since the discovery of the SOD1 mutation in ALS, oxidative stress has been implicated in the pathogenesis of ALS. Although the exact mechanisms by which mutation in SOD1 result in motor neuron degeneration remain unclear, it has been suggested that the toxicity of the mutant protein is attributed to the gain of a novel toxic function of the protein, rather than to the reduction of superoxide dismutase activity [3,4].

Clinical data and the studies in transgenic ALS mice support the role of oxidative damage in the pathogenesis of ALS. Increased oxidative damage to proteins has been shown in the cortex of SALS and ALS patients [5,6] and in the spinal cord of SALS patients [7], as well as in the spinal cord of transgenic mice with the G93A SOD1 mutation [8]. Levels of 3-nitrotyrosine and of 3-nitro-4-hydroxyphenylacetic acid were found to be elevated in the spinal cord from FALS and SALS patients [9] and levels of 3-nitrotyrosine have been shown to increase in

Correspondence: M. Flint Beal, Department of Neurology and Neuroscience, Weill Medical College, Cornell University, 525 East 68th Street, A-501, New York, NY 10021. Tel: 212 746-6575. Fax: 212 746-8532. E-mail: fbeal@med.cornell.edu

ISSN 1071-5762 print/ISSN 1029-2470 online © 2005 Taylor & Francis Group Ltd
DOI: 10.1080/10715760400027979

cerebrospinal fluid (CSF) from SALS patients [10]. 3-nitrotyrosine levels were also found to be increased in SOD1 transgenic mice models of ALS [6,11]. Levels of 4-hydroxynonenal, a marker of lipid peroxidation, were elevated in CSF from SALS patients [12], and increased modification of proteins by 4-hydroxynonenal in spinal cord from SALS patients was demonstrated [13]. Additionally, overexpression of mutant SOD1 (G37R and G85R) in NT-2 cells increased levels of 4-hydroxynonenal, protein carbonyls, and 3-nitrotyrosine [14].

In addition to oxidative damage to proteins and lipids, increased oxidative damage to DNA in ALS has been demonstrated. 8-Hydroxy-2′-deoxyguanosine (8OH2′dG) levels have been found to increase in DNA isolated from the spinal cord [15] and from the motor cortex from SALS patients [6]. We found that free levels of 8OH2′dG are increased in urine, plasma and CSF from both SALS and FALS patients; plasma and urine 8OH2′dG levels increased with the progression of the disease and the rate of increase in urinary 8OH2′dG with time correlated with the disease severity [16]. Using an immunocytochemical approach progressive accumulation of 8OH2′dG in spinal cord from G93A mice has been demonstrated [17].

In the present study we investigated oxidative damage to DNA in transgenic ALS mice with the G93A SOD1 mutation. We examined 8OH2′dG levels in the nuclear DNA isolated from the spinal cord, frontal cortex, striatum and cerebellum from G93A mice at different time points of the disease: 60 days (presymptomatic stage), 90 days (symptomatic stage with early loss of motor neurons), and 120 days (terminal stage). Furthermore, we used *in vivo* microdialysis to measure free levels of 8OH2′dG and 8OHG at the same time points in the frontal cortex of G93A mice.

Materials and Methods

Mice

Transgenic male mice carrying a mutant human SOD1 with a $Gly^{93} \rightarrow Ala$ (G93A) mutation were used in the experiments. Age-matched littermate mice were used as controls. 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.

Microdialysis

Concentric microdialysis probes (membrane length 2 mm, o.d. 200 μm; MW cutoff 18 KDa, Spectrum Inc., Houston, TX; recovery for 8OH2′dG and 8OHG *in vitro* 6–8%) were implanted into the right frontal cortex (coordinates from bregma: AP +2.4, ML 1.2, V—3.2) 20–24 h before the perfusion experiments. The perfusion medium (145 mM NaCl, 2.7 mM KCl, 1.0 mM $MgCl_2$, 1.2 mM $CaCl_2$) was delivered at 5 μl/min (CMA/102 microdialysis pump, CMA Microdialysis, Chelmsford, MA). After at least 1 h equilibration period, microdialysates were collected for the next 10 h. Due to the very low levels of 8OH2′dG and 8OHG in the brain extracellular fluid the entire resulting volumes were concentrated using solid phase extraction (SPE) protocol to obtain reliable measurements of the analytes. C18 500 mg SPE columns (Diazem Corporation, Midland, MI) were preconditioned sequentially with 1 ml of 100% methanol (MeOH), 2 ml of water and 1 ml of the perfusion medium. Microdialysis samples were loaded onto the column followed by 1 ml of 4% MeOH and the entire resulting volumes were collected (8OHG-containing fraction). 8OH2′dG was then eluted using 1.3 ml of 27% acetonitrile (8OH2′dG-containing fraction). All elution steps were done under gravity flow. The fractions were evaporated under vacuum centrifugation and reconstituted in 100 μl of mobile phase. Aliquots of 80 μl were analyzed.

Isolation and digestion of DNA

After completion of the microdialysis perfusion the animals were sacrificed and spinal cord, striatum, frontal cortex (on the intact hemisphere), and cerebellum were dissected on ice. The tissues were homogenized and genomic DNA was isolated by a chaotropic NaI method using a DNA Extractor Kit (Wako Chemicals, Richmond, VA). Extracted DNA was dissolved in 100 μl of 10 mM Trizma base, pH 7.5 and incubated with 7 μl of nuclease P1 (Calbiochem Inc., La Jolla, CA) (1100U/ml in 25 mM sodium acetate, containing 1 mM ZnCl2, pH 4.8) at 37°C for 60 min. Following the addition of 6 μl of alkaline phosphatase (Boehringer-Mannheim GmbH, Mannheim, Germany) (750U/ml in 100 mM Tris-HCl, pH 8.0), the samples were incubated at 37°C for 30 min. Aliquots of 80 μl were used for analysis.

Sample analysis

Free levels of 8OH2′dG and 8OHG in microdialysis samples and 2′-deoxyguanosine (2′dG) and 8OH2′dG in DNA were measured using a carbon column-based HPLC method with electrochemical detection, essentially as described elsewhere [18,19]. Statistical comparisons were made by non-paired Student's *t*-test with Bonferroni correction.

Results

Levels of 8OH2′dG in DNA from the spinal cord of G93A mice were significantly higher, as compared to

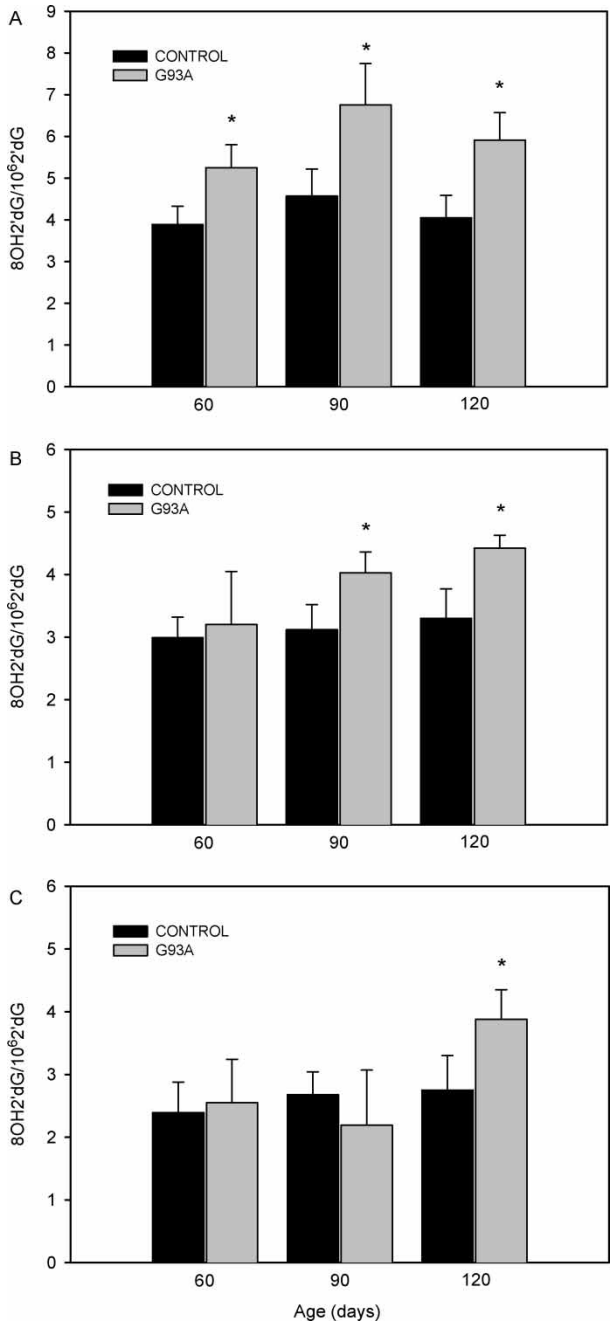

Figure 1. 8OH2′dG levels (expressed per 10^6 2′dG) in DNA isolated from the spinal cord (A), the frontal cortex (B), and the striatum (C) of G93A mice and age-matched littermate control mice at 60, 90 and 120 days of age. Data are expressed as mean ± SEM. $^*p < 0.05$. $n = 6–8$ per group.

controls at all time points studied (Figure 1A). At 60, 90 and 120 days of age levels of 8OH2′dG were increased by 35, 49, and 45%, respectively. 8OH2′dG levels were also significantly increased in DNA from the frontal cortex of G93A mice, as compared to controls at 90 and 120 days, by 29 and 34%, respectively (Figure 1B). There were no significant changes in 8OH2′dG in DNA from the striatum of G93A mice at 60 and 90 days of age. However, levels of 8OH2′dG in striatal DNA at 120 days were significantly higher (by 41%) in G93 mice than in controls (Figure 1C). No significant changes in 8OH2′dG levels in DNA extracted from cerebellum were found at any time point between the G93A and the control mice. In G93A mice the levels of 8OH2′dG (expressed per 10^6 2′dG) at 60, 90 and 120 days of age were (mean ± SEM): 6.33 ± 0.65, 7.17 ± 0.83, and 7.03 ± 0.49, respectively ($n = 6–8$ per group). In control mice the levels of 8OH2′dG (expressed per 10^6 2′dG) at 60, 90 and 120 days of age were (mean ± SEM): 7.06 ± 0.86, 7.49 ± 0.73, and 6.38 ± 0.91, respectively ($n = 6–8$ per group).

In addition to the levels of 8OH2′dG in DNA extracted from the spinal cord and different brain structures, we measured free levels of 8OH2′dG and 8OHG in frontal cortical extracellular fluid of G93A and control mice, using *in vivo* microdialysis. Free levels of 8OH2′dG in cortical microdialysates of G93A mice were significantly increased, as compared to controls, at 90 and 120 days of age, by 46 and 33%, respectively (Figure 2A). At 120 days

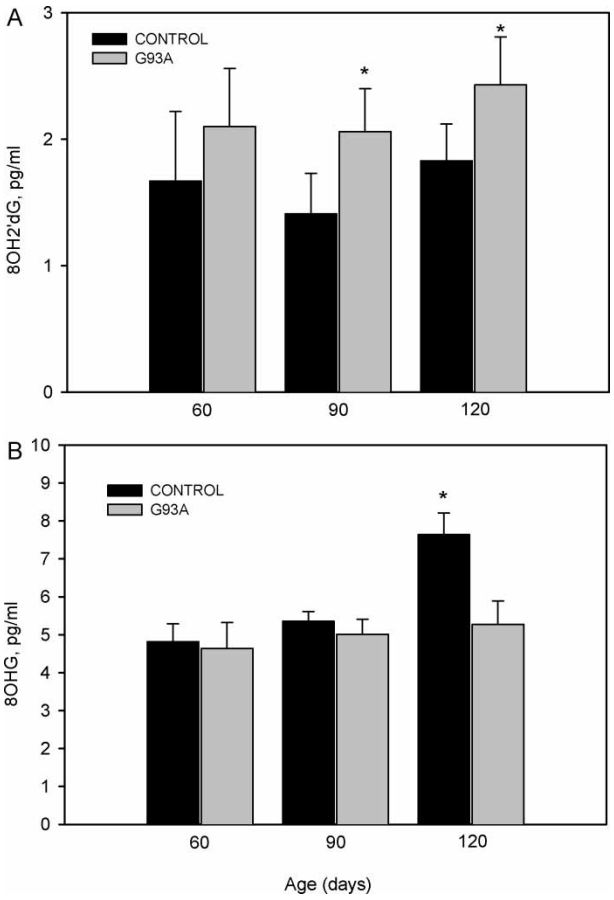

Figure 2. Free levels of 8OH2′dG (A) and 8OHG (B) in the frontal cortical microdialysates in G93A and age-matched littermate control mice at 60, 90 and 120 days of age. Data are expressed as mean ± SEM. $^*p < 0.05$. $n = 6–8$ per group.

free levels of 8OHG in the frontal cortical microdialysates in control mice were 45% higher than those in G93A mice (Figure 2B).

Discussion

In the present study we found that in the G93A mice, 8OH2′dG levels in the nuclear DNA are increased in the spinal cord (at 60, 90 and 120 days), in the frontal cortex (at 90 and 120 days), and in the striatum (at 120 days), as compared to age-matched littermate controls. No significant changes in the levels of 8OH2′dG isolated from the cerebellum were observed at any of the time points studied. Furthermore, using *in vivo* microdialysis we demonstrated an increase in the levels of free 8OH2′dG in the cortex of G93A mice, as compared to control mice, at 90 and 120 days. In contrast to 8OH2′dG, free levels of 8OHG were significantly higher in control mice than in G93A mice at 120 days of age.

There is a substantial evidence that oxidative stress is implicated in the pathogenesis of ALS. Several previous reports showed that markers of oxidative damage to proteins [6,19], lipids [13], and DNA [6,15] are increased in ALS. Our findings are consistent with previous reports showing, both in ALS patients and transgenic ALS animal models, an increase in oxidative damage to DNA [6,15–17,20,21]. Other evidence implicating oxidative damage in disease pathogenesis comes from studies showing that crossing ALS transgenic mice with mice partially deficient in manganese SOD exacerbates the disease phenotype. Furthermore, catalytic antioxidants significantly prolong the survival of G93A SOD in mice [22,23]. Treatment of G93A SOD1 mice with the free radical spin trap DMPO also significantly increases survival and delays paralysis [24]. An increase in free radical generation in spinal cord of G93A SOD mice was demonstrated with a novel brain permeable azulenyl nitrone [25]. The present results provide direct evidence for the oxidative DNA damage in the spinal cord and the frontal cortex, the areas undergoing motor neuron degeneration during the disease. No changes in oxidative DNA damage were found in the cerebellum. The levels of 8OH2′dG in DNA were significantly increased in the striatum at the terminal stage of the disease (120 days), although no striatal pathology has been described in G93A mice. However, we previously found that in G93A SOD1 mouse model of ALS the rate of hydroxyl radical production *in vivo* is increased in the striatum at 90 days of age [26]. Furthermore, these mice show increased vulnerability to striatal lesions produced by 3-nitropropionic acid [27]. These observations suggest that biochemical changes may precede observable pathological changes in these mice.

The C-8 hydroxylation of guanine is one of the most frequent base modifications following oxidative stress [28,29]. This lesion is generated by either direct oxidation by hydroxyl radical, or could result from a "hydroxyl radical-like process" involving peroxynitrite [30,31]. 8OH2′dG is the most abundant of the described oxidative DNA lesions [32] and could be the most critical one, because of its higher propensity for mispairing with adenosine, rather than cytosine during replication [33,34]. When there is insufficient DNA repair this results in an increased frequency of G to T transversion mutations [35,36]. Base excision-repair (BER) is the major pathway for removal of oxidative DNA lesions, including those of guanine [37]. In the case of oxidative guanine lesions, BER is initiated by 8OHG-DNA glycosylase (OGG1) which excises the base lesion (as 8OHG) from DNA [38–41]. Although BER is a major repair pathway, nucleotide excision-repair (NER) has been shown to participate in the repair of oxidative guanine lesions, yielding 8OH2′dG [42–44]. In *ogg1* null knockout mice, despite massive accumulation of 8OH2′dG in DNA, there is slow but significant removal of the oxidized base [45].

In this study we observed that both free levels of 8OH2′dG and the levels of 8OH2′dG lesion in DNA are increased in G93A, mice as compared to control mice. On the other hand, while levels of 8OH2′dG, both free and in DNA, were unchanged in control mice, there was a significant increase in free levels of 8OHG in the frontalcortex of control mice at 120 days of age, as compared to G93A mice. There is evidence for increased oxidative DNA damage with aging (see [46] for the review). We previously found that critical levels of 8OH2′dG increase with aging in human brain, and the increase is most marked in mitochondrial DNA [47]. We also observed age-dependent increases in 8OH2′dG in human skeletal muscle [48]. Furthermore, we found age-dependent increases in 8OH2′dG in human plasma and CSF [16]. Therefore, an increase in free levels of 8OHG despite no change in 8OH2′dG levels in DNA from control mice suggest that, despite an age-dependent increase in endogenous oxidative damage to DNA, it is efficiently repaired by BER. In support of this, in senescence-accelerated phenotype SAMP1 and SAMP8 mice increased levels in 8OH2′dG in whole brain DNA are associated with the impaired activity of OGG1 [49]. Increased levels of 8OH2′dG in DNA without corresponding increase in free levels of 8OHG in G93A mice strongly suggest that BER may be impaired in these mice, while elevated levels of free 8OH2′dG in G93A mice most likely reflect a compensatory increase in the activity of NER. Although currently direct evidence for impaired BER in ALS is not available, it has been reported recently that expression of Ref-1 protein in the spinal cord of G93A mice [50], and expression of OGG1 in the spinal cord of SALS patients [21] are decreased.

In conclusion, the present findings in G93A transgenic mouse model of ALS provide direct evidence that oxidative DNA damage is increased and that BER pathway of DNA repair maybe deficient.

Acknowledgements

This work was supported by the ALS Association.

References

[1] Brown RH, Jr. Amyotrophic lateral sclerosis: Recent insights from genetics and transgenic mice. Cell 1995;80:687–692.

[2] Rosen DR, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 1993;362:59–62.

[3] Gurney ME, et al. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. Science 1994;264:1772–1775.

[4] Wong PC, et al. An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. Neuron 1995;14:1105–1116.

[5] Bowling AC, et al. Superoxide dismutase concentration and activity in familial amyotrophic lateral sclerosis. J Neurochem 1995;64:2366–2369.

[6] Ferrante RJ, et al. Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis. J Neurochem 1997;69:2064–2074.

[7] Shaw IC, Fitzmaurice PS, Mitchell JD, Lynch PG. Studies on cellular free radical protection mechanisms in the anterior horn from patients with amyotrophic lateral sclerosis. Neurodegeneration 1995;4:391–396.

[8] Andrus PK, Fleck TJ, Gurney ME, Hall ED. Protein oxidative damage in a transgenic mouse model of familial amyotrophic lateral sclerosis. J Neurochem 1998;71:2041–2048.

[9] Beal MF, et al. Increased 3-nitrotyrosine in both sporadic and familial amyotrophic lateral sclerosis. Ann Neurol 1997;42:644–654.

[10] Tohgi H, et al. Remarkable increase in cerebrospinal fluid 3-nitrotyrosine in patients with sporadic amyotrophic lateral sclerosis. Ann Neurol 1999;46:129–131.

[11] Bruijn LI, et al. Elevated free nitrotyrosine levels, but not protein-bound nitrotyrosine or hydroxyl radicals, throughout amyotrophic lateral sclerosis (ALS)-like disease implicate tyrosine nitration as an aberrant *in vivo* property of one familial ALS-linked superoxide dismutase 1 mutant. Proc Natl Acad Sci USA 1997;94:7606–7611.

[12] Smith RG, Henry YK, Mattson MP, Appel SH. Presence of 4-hydroxynonenal in cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis. Ann Neurol 1998;44:696–699.

[13] Pedersen WA, et al. Protein modification by the lipid peroxidation product 4-hydroxynonenal in the spinal cords of amyotrophic lateral sclerosis patients. Ann Neurol 1998;44:819–824.

[14] Lee M, Hyun DH, Halliwell B, Jenner P. Effect of overexpression of wildtype and mutant Cu/Zn-superoxide dismutases on oxidative stress and cell death induced by hydrogen peroxide, 4-hydroxynonenal or serum deprivation: Potentiation of injury by ALS-related mutant superoxide dismutases and protection by Bcl-2. J Neurochem 2001;78:209–220.

[15] Fitzmaurice PS, et al. Evidence for DNA damage in amyotrophic lateral sclerosis. Muscle Nerve 1996;19:797–798.

[16] Bogdanov M, et al. Increased oxidative damage to DNA in ALS patients. Free Radic Biol Med 2000;29:652–658.

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

[18] Bogdanov MB, Beal MF, McCabe DR, Griffin RM, Matson WR. A carbon column-based liquid chromatography electrochemical approach to routine 8-hydroxy-2′-deoxyguanosine measurements in urine and other biologic matrices: A one-year evaluation of methods. Free Radic Biol Med 1999;27:647–666.

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

[20] Liu D, Wen J, Liu J, Li L. The roles of free radicals in amyotrophic lateral sclerosis: Reactive oxygen species and elevated oxidation of protein, DNA, and membrane phospholipids. Faseb J 1999;13:2318–2328.

[21] Kikuchi H, et al. Impairment of mitochondrial DNA repair enzymes against accumulation of 8-oxo-guanine in the spinal motor neurons of amyotrophic lateral sclerosis. Acta Neuropathol (Berl) 2002;103:408–414.

[22] Jung C, et al. Synthetic superoxide dismutase/catalase mimetics reduce oxidative stress and prolong survival in a mouse amyotrophic lateral sclerosis model. Neurosci Lett 2001;304:157–160.

[23] Wu AS, et al. Iron porphyrin treatment extends survival in a transgenic animal model of amyotrophic lateral sclerosis. J Neurochem 2003;85:142–150.

[24] Liu R, et al. Increased mitochondrial antioxidative activity or decreased oxygen free radical propagation prevent mutant SOD1-mediated motor neuron cell death and increase amyotrophic lateral sclerosis-like transgenic mouse survival. J Neurochem 2002;80:488–500.

[25] Liu R, Althaus JS, Ellerbrock BR, Becker DA, Gurney ME. Enhanced oxygen radical production in a transgenic mouse model of familial amyotrophic lateral sclerosis. Ann Neurol 1998;44:763–770.

[26] Bogdanov MB, Ramos LE, Xu Z, Beal MF. Elevated "hydroxyl radical" generation *in vivo* in an animal model of amyotrophic lateral sclerosis. J Neurochem 1998;71:1321–1324.

[27] Andreassen OA, et al. Transgenic ALS mice show increased vulnerability to the mitochondrial toxins MPTP and 3-nitropropionic acid. Exp Neurol 2001;168:356–363.

[28] Kasai H. Analysis of a form of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. Mutat Res 1997;387:147–163.

[29] Floyd RA, Watson JJ, Harris J, West M, Wong PK. Formation of 8-hydroxydeoxyguanosine, hydroxyl free radical adduct of DNA in granulocytes exposed to the tumor promoter, tetradecanoylphorbolacetate. Biochem Biophys Res Commun 1986;137:841–846.

[30] Inoue S, Kawanishi S. Oxidative DNA damage induced by simultaneous generation of nitric oxide and superoxide. FEBS Lett 1995;371:86–88.

[31] Spencer JP, et al. Base modification and strand breakage in isolated calf thymus DNA and in DNA from human skin epidermal keratinocytes exposed to peroxynitrite or 3-morpholinosydnonimine. Chem Res Toxicol 1996;9:1152–1158.

[32] Dizdaroglu M, Olinski R, Doroshow JH, Akman SA. Modification of DNA bases in chromatin of intact target human cells by activated human polymorphonuclear leukocytes. Cancer Res 1993;53:1269–1272.

[33] Shibutani S, Takeshita M, Grollman AP. Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. Nature 1991;349:431–434.

[34] Maki H, Sekiguchi M. MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. Nature 1992;355:273–275.
[35] Harwood J, Tachibana A, Meuth M. Multiple dispersed spontaneous mutations: A novel pathway of mutation in a malignant human cell line. Mol Cell Biol 1991;11:3163–3170.
[36] Kamiya H, et al. 8-Hydroxyguanine (7,8-dihydro-8-oxoguanine) in hot spots of the c-Ha-ras gene: Effects of sequence contexts on mutation spectra. Carcinogenesis 1995;16:883–889.
[37] Krokan HE, Standal R, Slupphaug G. DNA glycosylases in the base excision repair of DNA. Biochem J 1997;325 (Pt 1):1–16.
[38] Nash HM, et al. Cloning of a yeast 8-oxoguanine DNA glycosylase reveals the existence of a base-excision DNA-repair protein superfamily. Curr Biol 1996;6:968–980.
[39] Mitra S, et al. Complexities of DNA base excision repair in mammalian cells. Mol Cells 1997;7:305–312.
[40] Hazra TK, Izumi T, Maidt L, Floyd RA, Mitra S. The presence of two distinct 8-oxoguanine repair enzymes in human cells: Their potential complementary roles in preventing mutation. Nucleic Acids Res 1998;26:5116–5122.
[41] Hazra TK, et al. Characterization of a novel 8-oxoguanine-DNA glycosylase activity in *Escherichia coli* and identification of the enzyme as endonuclease VIII. J Biol Chem 2000;275:27762–27767.
[42] Reardon JT, Bessho T, Kung HC, Bolton PH, Sancar A. *In vitro* repair of oxidative DNA damage by human nucleotide excision repair system: Possible explanation for neurodegeneration in xeroderma pigmentosum patients. Proc Natl Acad Sci USA 1997;94:9463–9468.
[43] Dianov G, Bischoff C, Piotrowski J, Bohr VA. Repair pathways for processing of 8-oxoguanine in DNA by mammalian cell extracts. J Biol Chem 1998;273:33811–33816.
[44] Gros L, Saparbaev MK, Laval J. Enzymology of the repair of free radicals-induced DNA damage. Oncogene 1999;21:8905–8925.
[45] Klungland A, et al. Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. Proc Natl Acad Sci USA 1999;96:13300–13305.
[46] Floyd RA, Hensley K. Oxidative stress in brain aging. Implications for therapeutics of neurodegenerative diseases. Neurobiol Aging 2002;23:795–807.
[47] Mecocci P, et al. Oxidative damage to mitochondrial DNA shows marked agedependent increases in human brain. Ann Neurol 1993;34:609–616.
[48] Mecocci P, et al. Age-dependent increases in oxidative damage to DNA, lipids, and proteins in human skeletal muscle. Free Radic Biol Med 1999;26:303–308.
[49] Choi JY, et al. Thermolabile 8-hydroxyguanine DNA glycosylase with low activity in senescence-accelerated mice due to a single-base mutation. Free Radic Biol Med 1999;27:848–854.
[50] Nagano I, Murakami T, Manabe Y, Abe K. Early decrease of survival factors and DNA repair enzyme in spinal motor neurons of presymptomatic transgenic mice that express a mutant SOD1 gene. Life Sci 2002;72:541–548.