Free Radical Biology & Medicine, Vol. 28, No. 5, pp. 779–785, 2000

0891-5849/00/$–see front matter

PII S0891-5849(00)00172-6

Original Contribution

DNA DAMAGE, REPAIR, AND ANTIOXIDANT SYSTEMS IN BRAIN REGIONS: A CORRELATIVE STUDY

FERNANDO CARDOZO-PELAEZ,*,† PHILIP J. BROOKS,‡ TODD STEDEFORD,†,§ SHIJIE SONG,*,† and JUAN SANCHEZ-RAMOS*,†

*James Haley Veterans Affairs Research Service and †Department of Neurology and §College of Public Health, University of South Florida, Tampa, FL; and ‡Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Rockville, MD, USA

(*Received* 15 *September* 1999; *Revised* 09 *December* 1999; *Accepted* 11 *January* 2000)

Abstract—8-Hydroxy-2′-deoxyguanosine (oxo^{8}dG) has been used as a marker of free radical damage to DNA and has been shown to accumulate during aging. Oxidative stress affects some brain regions more than others as demonstrated by regional differences in steady state oxo^{8}dG levels in mouse brain. In our study, we have shown that regions such as the midbrain, caudate putamen, and hippocampus show high levels of oxo^{8}dG in total DNA, although regions such as the cerebellum, cortex, and pons and medulla have lower levels. These regional differences in basal levels of DNA damage inversely correlate with the regional capacity to remove oxo^{8}dG from DNA. Additionally, the activities of antioxidant enzymes (Cu/Zn superoxide dismutase, mitochondrial superoxide dismutase, and glutathione peroxidase) and the levels of the endogenous antioxidant glutathione are not predictors of the degree of free radical induced damage to DNA in different brain regions. Although each brain region has significant differences in antioxidant defenses, the capacity to excise the oxidized base from DNA seems to be the major determinant of the steady state levels of oxo^{8}dG in each brain region.

Keywords—DNA damage, DNA repair, 8-Hydroxy-2′-deoxyguanosine, Brain regions, Oxyradicals, Antioxidants, Free radicals

INTRODUCTION

Free radicals are formed in the central nervous system (CNS) as part of normal metabolic processes [1]. High oxygen uptake and low antioxidant defenses increase the vulnerability of the CNS to oxidative damage [2]. Assessment of free radical–induced damage to biological systems has been carried out by measuring the accumulation of damage to macromolecules. Proteins, lipids, and DNA are major targets for free radical–induced damage. Carbonyl content as an estimate of oxidative damage to proteins has been found to be high in specific brain regions and to be elevated during aging and in some types of neurodegenerative disorders [3,4]. Levels of malondialdehyde and 4-hydroxynonenal have been used to identify oxidative damage to lipids and its association with aging and disease [5,6]. The damage to DNA is more variable, and attack by free radicals can produce structural damage (i.e., strand breaks) and/or modification of the bases. 8-Hydroxy-2′-deoxyguanosine (oxo^{8}dG) is one of the most common adducts formed from the reaction of oxyradicals with DNA [7]. A relation between oxidative DNA damage and aging has been postulated based on studies that show inverse associations between steady state tissue levels of oxo^{8}dG and the life spans of different animal species [8,9]. There are also age-dependent increases in oxo^{8}dG levels in specific rat organs and the human brain [10,11]. We have recently shown that oxo^{8}dG accumulates differentially across brain regions in the aging mouse [12]. Oxo^{8}dG levels have been found to be high in regions involved in neurodegenerative diseases, which is suggestive of an accelerated aging process in specific populations of neurons [13–15].

Unrepaired DNA lesions might impair transcription and protein synthesis [16]. This type of damage would be particularly deleterious for terminally differentiated cells.

Cells have a complete set of antioxidant defenses that

Address correspondence to Juan Sanchez-Ramos, M.D., Ph.D., Department of Neurology, MDC 55, 12901 Bruce B. Downs Boulevard, Tampa, FL 33620, USA; Tel: (813) 974-6022; Fax: (813) 974-7200; E-Mail: jsramos@com1.med.usf.edu.

guard against the damaging effects of free radicals and a DNA repair system that fixes damage done by unscavenged free radicals. Endogenous antioxidants such as glutathione (GSH) and enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase are the first line of defense and act by scavenging potentially damaging free radical moieties. DNA repair systems involve a number of enzymatic processes ranging from base recognition and excision to ligation of DNA strands. The DNA glycosylases recognize a particular damaged base and remove the base by cutting the N-glycosylic bond of the DNA molecule. A bacterial enzyme that recognizes modified purines (including oxo^{8}dG) is formamidopyrimidine DNA glycosylase (Fpg) [17]. A similar activity has been described in mammals, and the enzyme responsible for oxo^{8}dG excision has been cloned and called 8-oxoguanine DNA glycosylase (Ogg1) [18,19].

Different regional activities in antioxidant systems and variable metabolic rates can lead to a region-specific accumulation of oxidative damage, and such differences can increase the vulnerability of specific brain regions to age-dependent oxidative stress. Several studies have been carried out to establish the age-dependent changes in activities of antioxidant enzymes [20–22]. It is hypothesized that decreased capacity in DNA repair and/or antioxidant defenses increases brain region vulnerability to oxidative damage and is responsible for age-associated deficits in cellular function, particularly in postmitotic cells such as neurons.

The overall objectives of this study were to measure steady state levels of oxo^{8}dG in specific brain regions as indices of regional oxidative DNA damage and to identify regional differences in oxo^{8}dG glycosylase activities and antioxidant defenses that could render specific brain regions more prone to oxidative DNA damage. This information will be useful in understanding the regional vulnerabilities of the brain during the normal process of aging, after vascular accidents, or elicited with exogenous toxicants.

MATERIALS AND METHODS

Measurement of oxo^{8}dG

Black male C57BL/N6 mice 3 months old were obtained from The Jackson Laboratories (Bar Harbor, ME, USA). Animals were killed by decapitation, and brains were place in ice-cold saline. Brain regions were dissected, and the samples were placed at −70°C until the time of assay. Procedures for extraction, purification, and enzymatic hydrolysis of DNA and measurement of oxo^{8}dG levels (detailed elsewhere [23]) were based on published methods [24,25] with minor modifications. Briefly, approximately 150 mg of brain tissue was used for extraction of sufficient DNA to perform the assay for oxo^{8}dG. The tissue was pulverized in liquid nitrogen using a mortar and pestle, homogenized in 10 mM of ethylenediaminetetraacetic acid (EDTA), and centrifuged. The supernatant was stored (at −70°C) for SOD and GPx assays. The pellet was treated with DNAase-free RNAase followed by digestion with proteinase K. The protein fraction was separated from DNA by three consecutive organic extractions. The DNA was then precipitated by adding two volumes of ethanol (with respect to the aqueous volume) and then incubated overnight at −20°C. The purity of the DNA was determined by the absorbance of an aliquot of the sample at 260 vs. 280 nm [24].

The purified DNA was prepared for high-performance liquid chromatography analysis by resolving it into deoxynucleoside components. The amount of oxo^{8}dG and 2-deoxyguanosine (2-dG) was calculated by comparing the peak area of oxo^{8}dG and 2-dG obtained from the enzymatic hydrolysate of the DNA sample with a calibration curve for both compounds. Levels of oxo^{8}dG in the samples were expressed relative to the content of 2-dG (e.g., the molar ratio of oxo^{8}dG to 2-dG [fmol of oxo^{8}dG/nmol of 2-dG]). Because 1 μg of DNA contains 0.648 nmol of 2-dG, 1 fmol/nmol of 2-dG is equivalent to 1.54 fmol/μg of DNA.

High-performance liquid chromatography system. The mobile phase was 100 mM of sodium acetate, pH 5.2, with 5% methanol. Oxo^{8}dG was detected by an electrochemical detector (ESA Coulochem Model 5100A, Chelmsford, MA, USA) using a glassy carbon-working electrode at an applied potential of +0.4 V. 2-dG was detected in the same sample by absorbance at 260 nm using a Perkin Elmer (Norwalk, CT, USA) 785A Programmable Absorbance Detector arranged in series with the electrochemical detector. Data were recorded, stored, and analyzed on a PC Pentium computer using ESA 500 Chromatography Data System software.

Measurement of SOD

SOD activity in brain regions was measured according to a previously described procedure based on the SOD-mediated inhibition of nitrite formation from hydroxylammonium in the presence of O_2^- generators [23, 26]. Aliquots of the first supernatant (from brain tissue homogenates prepared as described previously) were used for determination of SOD activity by incubating the homogenate with xanthine and hydroxylamine chloride. The reaction was initiated by the addition of xanthine oxidase. An aliquot of the incubation mixture was added to a mixture of sulfanilic acid and α-naphthylamine, and the absorbance was read at 529 nm (Ultrospec III spectrophotometer; Pharmacia LKB Biochrom, Cambridge, England).

To differentiate between mitochondrial SOD (MnSOD) and Cu/ZnSOD activity in the tissue homogenate, MnSOD was determined by the addition of potassium cyanide (5 mM) to the incubation medium for 20 min at room temperature. The difference between the total and potassium cyanide–inhibited enzyme activities was defined as Mn-SOD activity. The activity of SOD in the samples was determined from a calibration curve of the percentage of inhibition of nitrite formation versus SOD activity, which was constructed using known amounts of purified SOD containing 3500 U/mg of protein. One unit of enzyme activity was defined as the amount of SOD required to reduce cytochrome *c* by 50% in the coupled system with xanthine and xanthine oxidase at pH 7.8 at 25°C in a 3 ml reaction volume. The amount of protein in the samples was determined using the bicinchoninic acid (BCA) protein kit. Data are expressed as units of SOD activity per milligram of protein.

Measurement of GPx Activity

GPx activity was measured according to the method described by Wendel [27], with certain modifications. Briefly, a reaction cocktail was prepared in a vial containing 1 mg of β-nicotinamide adenine dinucleotide phosphate tetrasodium salt, (Sigma, St. Louis, MO, USA) containing the following: 9.2 ml of 1 mM of sodium azide in 50 mM of sodium phosphate buffer with 0.4 mM of EDTA (pH 7.0), 10 units of glutathione reductase in 0.1 ml of cold deionized water, and 2 mM of reduced glutathione in 0.05 ml of cold deionized water. The assay was performed using 3 ml of the reaction cocktail with the addition of 50 to 100 μl of brain tissue homogenate, mixed by inversion, and equilibrated to 25°C in a 1 cm light path cuvette. The absorbance was monitored at 340 nm until constant using an SLM Aminco (Rochester, NY, USA) DW-2000 UV-Vis Spectrophotometer. Then, 0.0007% (wt/wt) hydrogen peroxide was added and immediately mixed by inversion, and the decrease in absorbance at 340 nm was recorded every 15 seconds for 5 min. Specific activity was computed based on the millimolar extinction coefficient of 6.22 for β-nicotinamide adenine dinucleotide phosphate at 340 nm. One unit of GPx catalyzes the oxidation by H_2O_2 of 1 μM of reduced glutathione to oxidized glutathione per minute at pH 7.0 at 25°C.

Oxo^{8}dG Repair Activity

Mouse brain regions were dissected on ice and placed under liquid nitrogen, and DNA glycosylases were extracted from tissue following homogenization with buffer containing 20 mM of Tris (pH 8.0), 1 mM of EDTA, 1 mM of dithiothreitol, 0.5 mM of spermine, 0.5 mM of spermidine, 50% glycerol, and protease inhibitors. Homogenates were rocked for 30 min after the addition of a 1/10 vol/vol of 2.5 M of KCl and spun at 14,000 rpm for 30 min. The supernatant was aliquoted and stored at −70°C until the time of assay. Protein levels were determined using the BCA method.

Twenty picomoles of synthetic probe containing oxo^{8}dG (Trevigen, Gaithersburg, MD, USA) was labeled with P^{32} at the 5′ end using polynucleotide T4 kinase (Boehringer Mannheim, Germany). For the nicking reaction, protein extract (30 μg) was mixed with 20 μl of a reaction mixture containing 0.5 M of N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid], 0.1 M of EDTA, 5 mM of dithiothreitol, 400 mM of KCl, purified BSA, and labeled probe (approximately 2000 cpm). The reaction was carried out at 37°C for 2 h and stopped by placing the solution in ice. An aliquot of this reaction mixture was added to a loading buffer containing 90% formamide, 10 mM of NaOH, and blue-orange dye. After 5 min of heating at 95°C, samples were chilled and loaded into a polyacrylamide gel (20%) with 7 M of urea and 1× TBE and run at 400 mV for 2 h. Gels were quantified using a Biorad (Hercules, CA, USA) 363 phosphoimager system and analysis software. Activity to repair oxo^{8}dG was determined and expressed as a percentage of substrate cleaved.

GSH content

Total levels of GSH in each brain region analyzed were measured by the method of Tietz [28]. Tissue was homogenized in phosphate buffer (0.5 M, pH 7.2), and aliquots were added to a reaction mixture containing 2-nitro-5-thiobenzoic acid as a chromogenic substance. Absorbance was determined at 412 nm. The content of GSH in the tissue was determined by comparing its absorption with that of a curve made with known amounts of GSH. Data were expressed as nanomoles per milligram of protein.

Data analysis and statistics

One-way ANOVA was used to compare regions, followed by a student Newman Keuls test to compare differences in oxyradical adduct levels (as well as SOD, GPx, and repair activities). Linear regression analyses were carried out for the correlation studies. Statistical tests were performed with Graphpad statistical software (Sorrento, CA, USA). A probability value less than .05 was considered to be significant.

RESULTS

Before presenting a summary of results, which are based on well-known methods for measuring antioxidant

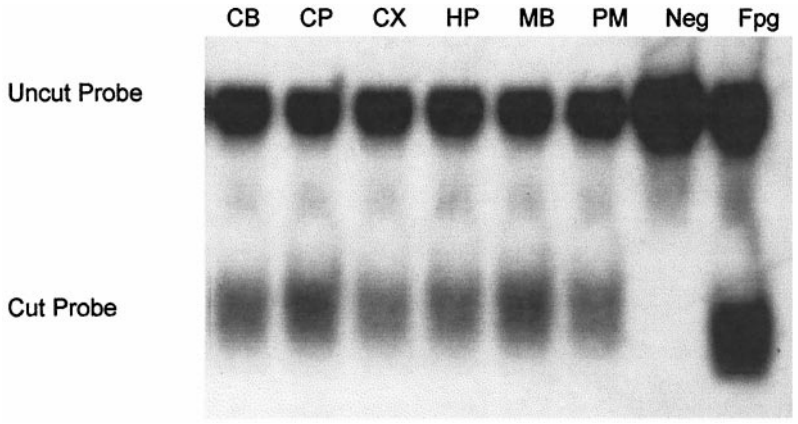

Fig. 1. Gel for the oxo[8]dG repair analysis in six brain regions (cerebellum [CB], caudate putamen [CP], cortex [CX], hippocampus [HP], midbrain [MB], and pons and medulla [PM]), followed by a negative control and a positive control (5 units of pure formamidopyrimidine glycosylase DNA glycosylase). Nuclear extracts from the brain regions were incubated with labeled probe containing oxo[8]dG as described in Materials and Methods. Protein extracts from four mice were run in duplicate under similar conditions.

enzyme activities and oxo[8]dG levels, the results on regional differences in DNA repair are detailed, because the method has not been applied previously to measurements in brain regions.

Measurement of oxidized DNA repair in specific brain regions was based on the capacity of nuclear extracts to cleave an oxo[8]dG-containing oligonucleotide. The repair activity of a specific brain region was determined by the analysis of gels as shown in Fig. 1. The repair activity was expressed as the ratio of the cut band (bottom) to the sum of the cut and uncut bands (top).

To determine the best concentration of protein required to analyze DNA repair, increasing concentrations of protein from nuclear extracts were run with the labeled oligonucleotide as described in Materials and Methods. Figure 2 shows a typical saturation protein curve. Near-maximal repair activity was achieved with the addition of 50 μg of protein. Thirty micrograms of protein was used to maintain the repair activity in the linear portion of the curve.

The main objective of this study was to compare the specific antioxidant capacities in different regions of the brain to the degree of oxidative DNA damage and DNA repair activity. Table 1 summarizes levels of enzyme activities, endogenous antioxidants, and steady state oxo[8]dG in each region studied. Two-way ANOVA analysis indicated that there were statistically significant differences in the values for the regions in all the parameters analyzed (Cu/ZnSOD, MnSOD, oxo[8]dG, GPx, GSH, and repair activity of oxo[8]dG).

Figure 3 is a composite of the analysis of the relations between antioxidant capacities and the levels of oxidative damage to DNA. Linear regression analysis indicated that there was a significant inverse correlation between the levels of oxo[8]dG and the corresponding regional capacity to repair this damage. Although high levels of MnSOD activity tend to correspond to low levels of oxo[8]dG, such analysis did not reach statistical significance. There was no correlation between activities of the other antioxidant enzymes, or of GSH, and levels of oxo[8]dG.

DISCUSSION

This article summarizes the region-specific differences in oxidative DNA damage and antioxidant capacities determined by levels of endogenous antioxidant (GSH), the activities of antioxidant enzymes (Cu/Zn-SOD, MnSOD, GPx), and the activity of the glycosylase responsible for oxo[8]dG removal. Our results demonstrate that low steady state oxo[8]dG repair activities in the adult mouse brain are inversely correlated with high steady state levels of oxidative DNA damage. In the 3 month old mouse, the highest level of oxo[8]dG repair activity

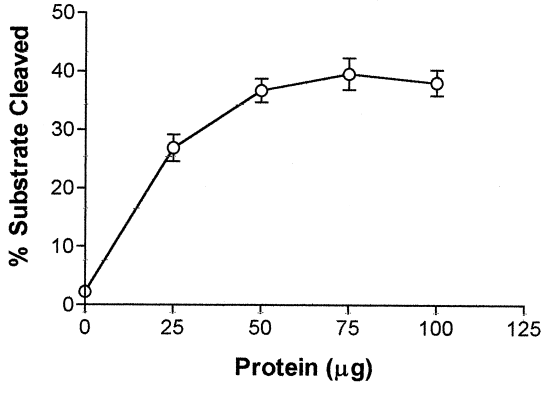

Fig. 2. Analysis of oxo[8]dG glycosylase activity with increasing amounts of protein from nuclear extracts. Each point represents the average of four different extracts run in duplicate. Data are expressed as the mean ± SEM.

Table 1. Regional Levels of Oxidative Damage to DNA and Antioxidant Systems

Region	Oxo^{8}dG (fmol.nmol 2-dg) (n = 7–8)	MnSOD U/mg of protein (n = 6)	CuZnSOD U/mg of protein (n = 6)	GPX U/mg of protein per min (n = 6)	GSH nmole/μg of protein (n = 5)	Oxo^{8}dG repair (% substrate cleaved) (n = 4)
Cerebellum	9.01 ± 0.81	0.17 ± 0.01	6.11 ± 0.37	11.49 ± 1.08	36.98 ± 1.96	20.33 ± 4.00
Caudate putamen	6.61 ± 0.97	0.37 ± 0.07	26.82 ± 3.52	19.46 ± 1.58	36.62 ± 2.38	36.01 ± 1.73
Cortex	10.68 ± 0.98	1.84 ± 0.19	21.20 ± 0.89	14.87 ± 0.58	37.21 ± 2.67	17.76 ± 1.64
Hippocampus	7.57 ± 0.96	2.78 ± 0.79	46.75 ± 7.57	9.61 ± 0.18	33.78 ± 1.04	34.40 ± 5.80
Midbrain	5.42 ± 1.21	2.88 ± 0.15	58.70 ± 1.68	18.60 ± 1.94	31.41 ± 1.43	33.26 ± 2.26
Pons and medulla	11.71 ± 2.11	0.51 ± 0.02	50.79 ± 1.71	17.09 ± 1.01	34.15 ± 1.01	20.68 ± 1.67

Data expressed as mean ± SEM.

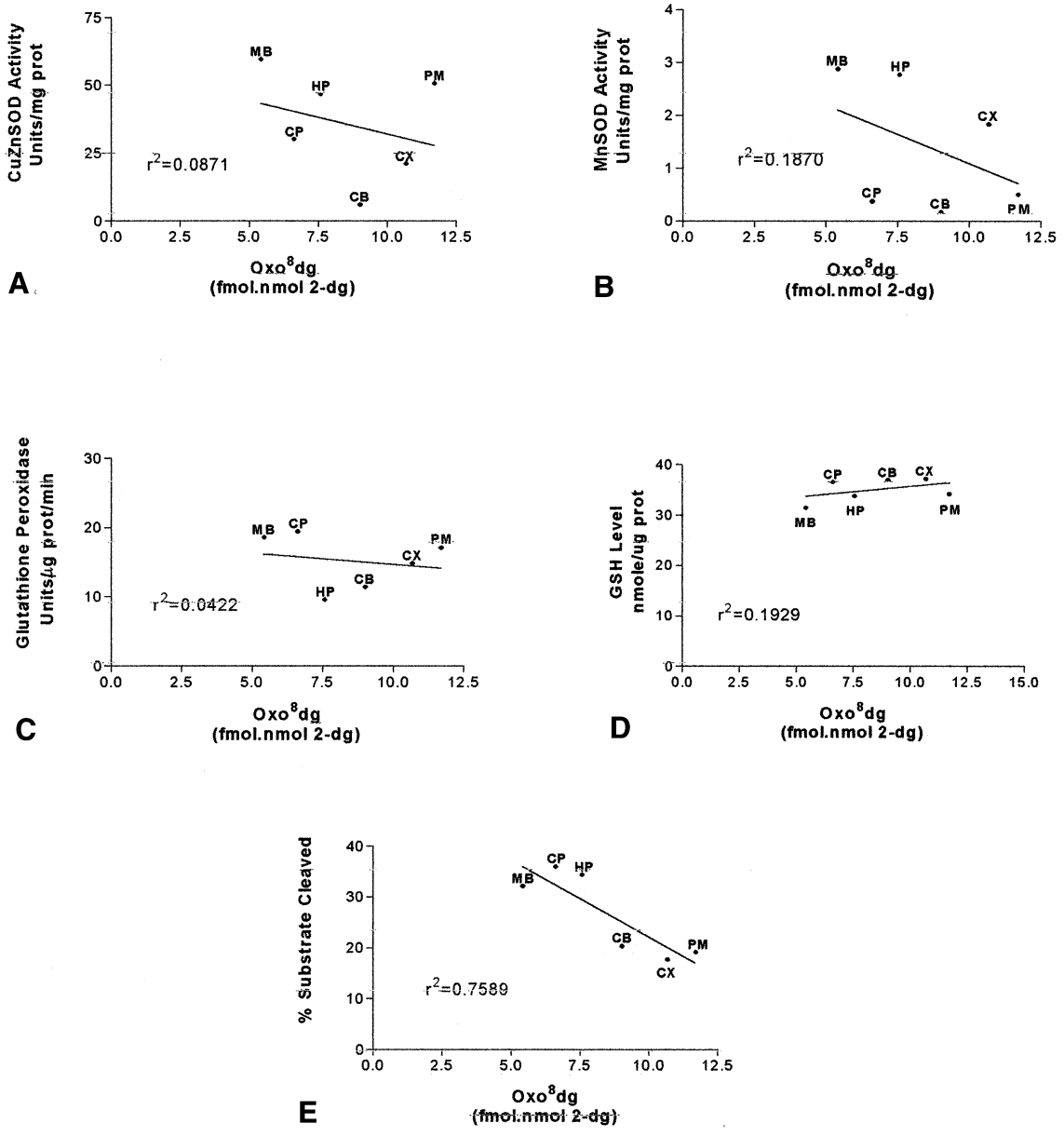

Fig. 3. Linear correlation analysis between regional oxo^{8}dG levels and antioxidant systems. (A) Cu/ZnSOD. (B) MnSOD. (C) GPx. (D) GSH. (E) Oxo^{8}dG repair activity.

was found in the caudate putamen, a region that showed the highest levels of oxidative DNA damage during aging or was associated with Parkinson's disease in other studies [11–13]. Because significant accumulation of DNA damage occurs in this region with age, it is inferred that DNA repair must decline with aging or in specific neurodegenerative diseases.

Accumulation of free radical damage as the cause of aging is supported by studies that show increased oxidatively induced protein damage in pathological conditions of accelerated aging in people and mice [29,30]. Other studies have demonstrated accumulation of free radical–induced damage in several organs during normal aging [31,32] or an increased susceptibility to free radical–induced damage as age increases [6,33]. A relation between DNA damage caused by free radicals and aging has been postulated based on studies that show an increase in the levels of oxo^{8}dG in most organs of aged rats and houseflies as well as in human brain tissue [9–11]. Additionally, an inverse relation exists between high urinary output of oxo^{8}dG and the life spans of various species of animals [8]. We have recently shown that oxo^{8}dG accumulates differentially across brain regions in the aging mouse [12]. That study showed an age-dependent increase in the oxo^{8}dG levels in the cerebellum, caudate putamen, and pons and medulla. The latter two regions have a high basal base excision repair capacity as shown by our present data, although the cerebellum exhibits one of the lowest repair activities at 3 months of age. Although these basal repair activities help to explain the accumulation of damage in individual regions, little is known of the changes that occur in DNA repair capacity with age in specific brain regions.

Evidence for the association between DNA oxidative damage and neurological deficits has been reported. There are higher steady state levels of oxo^{8}dG in the substantia nigra, basal ganglia, and cortex of patients with Parkinson's disease compared with controls [13]. The demise of dopaminergic neurons as a result of free radical damage has been postulated as a normal age-dependent phenomenon [31,34]. Recently, it was shown that during normal aging, oxo^{8}dG accumulated in regions involved in locomotion control and that these increased levels of oxo^{8}dG were associated with locomotion deficit [12]. Studies of hypersensitivity of cell lines to radiographs and ionizing radiation have provided evidence linking DNA repair deficiency to neurological disease [35,36]. Nevertheless, a more direct approach in determining repair capacity in the CNS could shed some light on the relation between defective DNA repair and neurological disease [37,38]. The role of an enzyme responsible for oxo^{8}dG repair in brain has not been well established. Although the activity of Ogg1 and the presence of its messenger RNA have been detected in several tissues by some investigators [19], others have failed to detect Ogg1 messenger RNA in mouse brain [18]. This could be because of a low level of Ogg1 message in the brain. Alternatively, it is possible that the oxo^{8}dG glycosylase activity we are detecting in brain extracts is distinct from Ogg1.

There was a trend, which did not reach statistical significance, towards an inverse relation between the regional activities of MnSOD and the levels of oxo^{8}dG. It has been shown that mitochondrial oxo^{8}dG levels are 15- to 16-fold higher than nuclear levels and that its levels accumulate with aging [11]. The data presented in this article represent the combination of nuclear and mitochondrial oxo^{8}dG, and this could allow for underestimation of the important role played by MnSOD in protecting mitochondrial DNA. Although it was previously believed that mitochondria did not possess oxo^{8}dG repair capacity [39], the presence of an enzyme capable of cleaving oxo^{8}dG has been demonstrated in liver and heart mitochondria [40,41]. Such activity is quite low compared with its nuclear counterpart, however.

A caveat to be noted is the complexity of the DNA base excision repair system. Our data are based on measurements of enzymatic activities of the base excision repair enzyme(s) responsible for the cleavage of oxo^{8}dG. Clearly, there are many other steps involved in base excision repair of damaged DNA in postmitotic tissues. The enzymatic components include the DNA N-glycosylases that recognize damaged purines and pyrimidines, the 5′ apurinic endonucleases that process strand breaks, sites of base loss, and the products of DNA glycosylase/apurinic lyase action as well as DNA polymerases and DNA ligases [42].

Although our data show that glycosylase activities vary significantly in different brain regions and are inversely related to steady state levels of oxo^{8}dG, there may be other components of DNA repair that are deficient in aging or specific neurodegenerative diseases.

In summary, we have found specific and significantly higher oxo^{8}dG repair activity in the brain regions that have been shown to be affected during aging and associated with various neurodegenerative diseases in other studies. These findings support the hypothesis that differential regional vulnerabilities to oxidative stress in normal aging or elicited by exogenous neurotoxicants are the result, in part, of intrinsic variations in the regulation of DNA repair mechanisms.

Acknowledgements — This study was supported by a Veterans Affairs Merit Review Grant and the Helen E. Ellis Research Fund at the University of South Florida.

REFERENCES

[1] Halliwell, B. Reactive oxygen species and the central nervous system. *J. Neurochem.* **5:**1609–1632; 1992.
[2] Harman, D. Free radical involvement in aging—pathophysiology and therapeutic implications. *Drugs Aging* **3:**60–80; 1993.
[3] Foster, M. J.; Dubey, A.; Dawson, K. M.; Stutts, W. A.; Lal, H.; Sohal, R. S. Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. *Proc. Natl Acad. Sci. USA* **93:**4765–4769; 1996.

[4] Floor, E.; Wetzel, M. G. W. Increased protein oxidation in human substantia nigra par compacta in comparison with basal ganglia and prefrontal cortex measured with an improved dinitrophenylhydrazine assay. *J. Neurochem.* **70:**268–275; 1998.
[5] Dexter, D. T.; Carter, C. J.; Wells, F. R.; Javoy-Agid, F.; Agid, Y.; Lees, A.; Jenner, P.; Marsden, C. D. Basal lipid peroxidation in *substantia nigra* is increased in Parkinson's disease. *J. Neurochem.* **52:**381–389; 1989.
[6] Cini, M.; Moretti, A. Studies on lipid peroxidation and protein oxidation in the aging brain. *Neurobiol. Aging* **16:**53–57; 1995.
[7] Dizdaroglu, M. Chemical determination of free radical-induced damage to DNA. *Free Radic. Biol. Med.* **10:**225–242; 1991.
[8] Adelman, R.; Saul, R. L.; Ames, B. N. Oxidative damage to DNA: relation to species metabolic rate and life span. *Proc. Natl. Acad. Sci. USA* **85:**2706–2708; 1988.
[9] Agarwal, S.; Sohal, R. S. DNA oxidative damage and life expectancy in houseflies. *Proc. Natl. Acad. Sci. USA* **91:**12332–12335; 1994.
[10] Fraga, C. G.; Shigenaga, M. K.; Park, J. W.; Degan, P.; Ames, B. N. Oxidative damage to DNA during aging: 8-hydroxy2′deoxyguanosine in rat organ DNA and urine. *Proc. Natl. Acad. Sci. USA* **87:**4533–4537; 1990.
[11] Mecocci, P.; MacGarvey, U.; Kaufman, A. E.; Koontz, D.; Shoffner, J. M.; Wallace, D. C.; Beal, M. F. Oxidative damage to mitochondrial DNA shows marked age-dependent increases in human brain. *Ann. Neurol.* **34:**609–616; 1993.
[12] Cardozo-Pelaez, F.; Song, S.; Parthasarathy, A.; Hazzi, C.; Naidu, K.; Sanchez-Ramos, J. Oxidative DNA damage in the aging mouse brain. *Mov. Disord.* **14:**972–980; 1999.
[13] Sanchez-Ramos, J.; Overvik, E.; Ames, B. N. A marker of oxyradical-mediated DNA damage (8-hydroxy-2′deoxyguanosine) is increased in nigro-striatum of Parkinson's disease brain. *Neurodegeneration* **3:**197–204; 1994.
[14] Gabbita, S. P.; Lovell, M. A.; Markesbery, W. R. Increased nuclear DNA oxidation in the brain in Alzheimer's disease. *J. Neurochem.* **71:**2034–2040; 1998.
[15] Lovell, M. A.; Gabbita, S. P.; Markesbery, W. R. Increased DNA oxidation and decreased levels of repair products in Alzheimer's disease ventricular CSF. *J. Neurochem.* **72:**771–776; 1999.
[16] Hatahet, Z.; Purmal, A. A.; Wallace, S. S. Oxidative DNA lesions as blocks to in vitro transcription by phage T7 RNA polymerase. *Ann. NY Acad. Sci.* **726:**346–348; 1994.
[17] Boiteux, S.; O'Connor, T. R.; Laval, J. Formamidopyrimidine-DNA glycosylase of *Escherichia coli*: cloning and sequencing of the *fpg* structural gene and overproduction of the protein. *EMBO J.* **6:**3177–3183; 1987.
[18] Rosenquist, T. A.; Zharkov, D. O.; Grollman, A. P. Cloning and characterization of a mammalian 8-oxoguanine DNA glycosylase. *Proc. Natl. Acad. Sci. USA* **94:**7429–7434; 1997.
[19] Radicella, J. P.; Dehrin, C.; Desmaze, C.; Fox, M. S.; Boiteux, S. Cloning and characterization of hOGG1, a human homolog of the *OGG1* gene of *Sacharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **94:**8010–8015; 1997.
[20] Cristiano, F.; de Haan, J. B.; Iannello, R. C.; Kola, I. Changes in the levels of enzymes which modulate the antioxidant balance occur during aging and correlate with cellular damage. *Mech. Ageing Dev.* **80:**93–105; 1995.
[21] Hussain, S.; Slikker, W.; Ali, S. F. Age-related changes in antioxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase and glutathione in different regions of mouse brain. *Int. J. Dev. Neurosci.* **13:**811–817; 1995.
[22] Haan, J. B.; Cristiano, F.; Iannello, R. C.; Kola, I. Cu/Zn-superoxide dismutase and glutathione peroxidase during aging. *Biochem. Mol. Biol. Int.* **35:**1281–1297; 1995.
[23] Cardozo-Pelaez, F.; Song, S.; Parthasarathy, A.; Epstein, C. J.; Sanchez-Ramos, J. Attenuation of age-dependent oxidative damage to DNA and protein in brainstem of Tg Cu/Zn SOD mice. *Neurobiol. Aging* **19:**311–316; 1998.
[24] Laws, G. M.; Adams, S. P. Measurement of 8-OHdG in DNA by HPLC/ECD: the importance of DNA purity. *Bioltech.* **20:**36–38; 1995.
[25] Shigenaga, M. K.; Park, J. W.; Cundy, K.; Gimenco, C. J.; Ames, B. N. In vivo oxidative DNA damage: measurement of 8-hydroxy-2′deoxyguanosine in DNA and urine by high performance liquid chromatography with electrochemical detection. *Methods Enzymol.* **186:**521–530; 1990.
[26] Elstner, E. F.; Heupel, A. Inhibition of nitrite formation from hydroxylammonium-chloride: a simple assay for superoxide dismutase. *Anal. Biochem.* **70:**616–620; 1976.
[27] Wendel, A. Glutathione peroxidase. *Methods Enzymol.* **77:**325–333; 1981.
[28] Tietz, F. Enzymatic method for the quantitative determination of nanogram amounts of total and oxidized glutathione: application to mammalian blood and other tissues. *Anal. Biochem.* **27:**502–522, 1969.
[29] Stadtman, E. R. Protein oxidation and aging. *Science* **257:**1220–1224; 1992.
[30] Butterfield, A. D.; Howard, B. J.; Yatin, S.; Allen, K. L.; Carney, J. M. Free radical oxidation of brain proteins in accelerated senescence and its modulation by N-*tert*-butyl-α-phenylnitrone. *Proc. Natl. Acad. Sci. USA* **94:**674–678; 1997.
[31] Dubey, A.; Foster, M. J.; Lal, H.; Sohal, R. S. Effect of age and caloric intake on protein oxidation in different brain regions and on behavioral functions of the mouse. *Arch. Biochem. Biophys.* **333:**189–197; 1996.
[32] Martinez, M.; Hernandez, A. I.; Matinez, N.; Ferrandiz, M. L. Age-related increase in oxidized proteins in mouse synaptic mitochondria. *Brain Res.* **731:**246–248; 1996.
[33] Sanjiv, A.; Sohal, R. S. Relationship between aging and susceptibility to protein oxidative damage. *Biochem. Biophys. Res. Commun.* **194:**1203–1206; 1993.
[34] Fahn, S.; Cohen, G. The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. *Ann. Neurol.* **32:**804–812; 1992.
[35] Chen, P.; Kidson, C.; Imray, F. P. Huntington's disease: implications of associated cellular radiosensitivity. *Clin. Genet.* **20:**331–336; 1981.
[36] Robbins, J. H.; Otsuka, F.; Tarone, R. E.; Polinsky, R. J.; Brumback, R. A.; Nee, L. E. Parkinson's disease and Alzheimer's disease: hypersensitivity to X rays in cultured cell lines. *J. Neurol. Neurosurg. Psychiatry* **48:**916–923; 1985.
[37] Brooks, P. J. Detection of excision nuclease in cell-free extracts from the adult mammalian brain. *Mutat. Res.* **408:**37–46; 1998.
[38] Brooks, P. J.; Marietta, C.; Goldman, D. DNA mismatch repair and DNA methylation in adult brain neurons. *J. Neurosci.* **16:**939–945; 1996.
[39] Clayton, D. A.; Doda, J. N.; Friedberg, B. C. The absence of a pyrimidine-dimer repair mechanism in mammalian mitochondria. *Proc. Natl. Acad. Sci. USA* **71:**2777–2781; 1974.
[40] Croteau, D. L.; ap Rhys, M. J.; Hudson, E. K.; Dianov, G. L.; Hansford, R. G.; Bohr, V. A. An oxidative damage-specific endonuclease from rat liver mitochondria. *J. Biol. Chem.* **272:**27338–27344; 1997.
[41] Souza-Pinto, N. C.; Croteau, D. L.; Hudson, E. K.; Hansford, R. G. H., Bohr, V. A. Age-associated increase in 8-oxo-deoxyguanosine glycosylase/AP lyase activity. *Nucleic Acids Res.* **27:**1935–1942; 1999.
[42] Wallace, S. S. Oxidative damage to DNA and its repair. In: Scandalios, J. G., eds. *Oxidative stress and the molecular biology of antioxidant defenses.* Cold Harbor, NY: Cold Spring Harbor Laboratory Press; 1997:49–89.