

8OHdG Levels in Brain Do Not Indicate Oxidative DNA Damage in Alzheimer's Disease

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TE KOPPELE, J. M., P. J. LUCASSEN, A. N. SAKKEE, J. G. VAN ASTEN, R. RAVID, D. F. SWAAB AND C. F. A. VAN BEZOOIJEN. 8OHdG levels in brain do not indicate oxidative DNA damage in Alzheimer's Disease. NEUROBIOL AGING 17(6) 819–826, 1996.—Accumulation of oxidative DNA damage has been proposed to underlie aging and neurodegenerative diseases such as Alzheimer's Disease (AD). The DNA adduct 8-hydroxy-2'-deoxyguanosine (8OHdG) is considered a good indicator of oxidative DNA damage. To investigate whether this type of DNA damage is involved in AD etiology, 8OHdG levels were determined in postmortem human brain tissue of controls and AD patients (in frontal, occipital, and temporal cortex and in hippocampal tissue). Parametric studies in rat revealed no influences of postmortem delay, repeated freezing/thawing or storage time. In human brain, approximately two 8OHdG molecules were present per 10⁵ 2'-deoxyguanosines. In AD patients and controls, 8OHdG-levels were not related to age, sex, or brain region. Also, no differences were found between controls and AD patients. It was concluded that 8OHdG in nuclear DNA, although present throughout the brain in fairly high amounts, does not accumulate with age, nor does it appear to be involved in AD. More detailed studies are required to extend this conclusion to other types of oxidative damage. Copyright © 1996 by Elsevier Science Inc.

Alzheimer's Disease: Aging DNA damage HPLC-ECD Oxygen radicals 8-Hydroxy-2'-deoxyguanosine
Human brain Cortex Hippocampus

ONE of the theories on the etiology of normal aging and Alzheimer's disease (AD) involves the accumulation of DNA damage (18,25,43). Oxidative damage to proteins and DNA occurs as a consequence of the generation of oxidants such as superoxide anions, hydrogen peroxide, and hydroxyl radicals, byproducts of normal cellular metabolism (1,9,15). Several changes in AD tissue, such as condensed chromatin (29), increased peroxidation (50), and membrane abnormalities (55) are consistent with the expected effects of reactive oxygen species. Furthermore, free oxygen radicals have been suggested to be involved in β -amyloidosis (17).

To maintain the integrity of the genome, cells are equipped with antioxidant defense mechanisms and DNA repair systems (15,18,21,45). It has been hypothesized that some neurodegenerative diseases may be related to elevated levels of DNA damage as a consequence of a defect in DNA repair or a decrease in antioxidant capacity (4,13,18,40,41,45,54). For instance, diminished DNA repair capacity was shown to be involved in Cockayne's syndrome (28,52) and AD (5,40,42,49). In addition, a twofold higher number of DNA single strand breaks was observed in the cortex of AD patients as compared to controls (36), indicating that DNA damage may indeed play a role in AD.

The brain is expected to be particularly prone to oxygen radical

damage (20,23) because of its relatively high oxygen consumption and the high concentrations of some trace metals, important catalysts of oxidative damage, in certain brain areas (7,10,11,19). Oxygen free radicals, together with metals like iron or copper, are known to cause peroxidation and subsequent DNA lesions such as the oxidatively modified nucleoside 8-hydroxy-2'-deoxyguanosine [8OHdG; (2,6,15,35)]. 8OHdG is considered to be a useful biomarker of oxidative DNA damage because its formation can also be induced by oxidative stress in vitro (14,47). In addition, 8OHdG has been shown to accumulate with age in several tissues including brain (16,24,26,34), and it can be measured relatively easily with high sensitivity by HPLC with electrochemical detection [HPLC-ECD; (22,31,46,47)].

In the present study, we describe methodological adjustments of existing HPLC-ECD assays to determine 8OHdG levels in small autopsy specimens of human brain tissue. Parametric investigations were performed with respect to storage time, postmortem delay, and repeated freezing/thawing of brain tissue on 8OHdG levels in brain DNA. Subsequently, the assay was applied on cortical areas and hippocampal tissue of Alzheimer patients and control subjects to determine whether 8OHdG levels do support involvement of oxidative DNA damage in AD etiology.

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METHOD

Brain Material

Human brain tissue was obtained via the rapid autopsy program of the Netherlands Brain Bank (coordinator, Dr. R. Ravid). Brain tissue for the present study was dissected from the superior frontal gyrus, superior occipital gyrus, the medial temporal gyrus and the hippocampus. The samples were taken from a group of eight controls (mean age: 68.2 ± 15.3 years), who had no history of neurological or psychiatric disorders, and of eight clinically diagnosed and neuropathologically confirmed Alzheimer's disease cases (mean age: 83.8 ± 9.9 years) (see Table 1 for details). Because the pH of the cerebrospinal fluids of both groups was identical (Table 1), no apparent differences in agonal state (38) between the two groups are present. Tissue was dissected fresh, frozen in liquid nitrogen and stored at -80°C .

The patients had been clinically assessed and the diagnosis of "probable AD" was assessed by first excluding other possible causes of dementia by history, physical examination, and laboratory tests. Clinical diagnoses of "probable AD" were based on the NINCDS-ADRDA criteria (32). All cases were neuropathologically confirmed using conventional histopathological stainings including Bodian, H&E, Congo red, and Alz-50. Diagnoses were based on the presence and distribution of plaques and tangles in the sections.

Isolation and Preparation of DNA Samples

RNase A, RNase T1, micrococcal endonuclease, spleen phosphodiesterase, and nuclease P1 were obtained from Boehringer

Mannheim (Almere, The Netherlands); proteinase K from Gibco BRL (Life Technologies Inc., Gaithersburg, MD). 8OHdG was kindly supplied by Dr. C. Richter, Zurich, Switzerland. Deoxynucleosides 2'-deoxyadenosine, 2'-deoxycytosine, 2'-deoxyinosine, 2'-deoxyguanosine (dG), and thymidine were obtained from Sigma, St. Louis, MO.

Approximately 50 mg of brain tissue was powdered in liquid nitrogen and further homogenized using a Polytron homogenizer in digestion buffer (10 mM Tris-HCl, 5 mM EDTA, 0.5% wt/vol sodium dodecyl sulphate; pH 7.8). After incubation with proteinase K (500 $\mu\text{g}/\text{ml}$ for 3 h at 38°C), 20% vol/vol potassium acetate (8 M) was added and the solution was gently mixed for 5 min. Subsequently, 1 volume of chloroform was added. After gentle mixing for 5 min, the mixture was centrifuged at $2000 \times g$ for 5 min at 4°C . From the aqueous layer, DNA was precipitated by addition of 2 vol of 100% ethanol (stored at -20°C for 30 min or overnight). After centrifugation (5 min, $800 \times g$, 4°C), the precipitate was washed with 70% vol/vol ethanol, and again centrifuged. After reconstitution of the pellet at 4°C in TE buffer (10 mM Tris-HCl, 0.1 mM EDTA; 500 $\mu\text{l}/50$ mg tissue), Tris-HCl (1M; pH 7.4) was added to a final concentration of 50 mM (50 $\mu\text{l}/\text{ml}$). Stock solutions of RNase A and T1 were preincubated at 37°C for 15 min. RNase treatment of DNA was performed at 37°C for 30 min at final concentrations of 200 μg RNase A/ml and 100 U RNase T1/ml. RNase-treated DNA was precipitated with 100% ethanol, washed with 70% vol/vol ethanol, and reconstituted in TE as described above.

The quality of the isolated DNA (before and after RNase treatment) was assessed spectrophotometrically (routinely, ratios of

TABLE 1
HUMAN BRAIN TISSUE USED

Pat Nr.	Sex	Age (years)	PMD (h:min)	BW (g)	pH	Clinicopathological Data
A) Control subjects						
91-118	m	59	6:00	1415	nd	Lung carcinoma, neuraxis radiation, trombo-emboli, pneumonia
91-120	f	73	4:25	975	5.8	Myocardial infarction
91-123	f	71	7:25	1135	7.2	Myocardial infarction, decompensatio cordis
91-124	m	38	7:00	1600	5.9	Endocarditis, sepsis, multiorgan failure, acute infections
91-125	m	61	5:40	1380	6.4	Adenocarcinoma, abdominal bleeding following stomach resection
92-029	m	79	5:10	1129	7.2	Decompensatio cordis, pneumonia
92-030	f	78	6:35	1084	7.0	Myocardial infarction, decompensatio cordis, pneumothorax
92-032	m	87	8:00	1262	7.1	Pancreatic carcinoma, lung infarction, liver metastases
Mean		68.2	6:17	1250	6.65	
SD		(15.3)	(1:12)	(210)	(0.6)	
B) Alzheimer patients						
90-038	f	84	2:30	1185	6.6	AD, pneumonia, sepsis, dehydration
90-056	f	86	3:45	1070	6.6	AD, bronchopneumonia
90-066	m	89	4:55	1488	6.2	AD
90-069	f	91	4:10	1008	6.5	AD
90-078	f	84	3:35	1389	6.8	AD, decompensatio cordis
92-019	m	60	4:40	1331	6.5	Presenile AD
92-020	f	89	2:55	1172	6.7	AD, dehydration, pneumonia
92-023	f	80	4:20	1007	6.8	AD
Mean		83.8*	4:00*	1150	6.58	
SD		(7.3)	(0:50)	(200)	(0.19)	

Abbreviations: Pat Nr, patient code; f, female; m, male; AD, Alzheimer's disease; PMD, postmortem delay; BW, brain weight; pH, pH of the cerebrospinal fluid; nd, not recorded.

* Significantly different from the control group ($p < 0.05$; unpaired t -test).

absorbance at 260 nm/280 nm were between 1.8 and 2.0) and by ethidium bromide/agarose gelelectrophoresis (1 μ g of DNA applied) (44). Rough quantitation of the amounts of DNA needed for DNA hydrolysis was performed spectrophotometrically (1 absorption unit at 260 nm corresponds to 50 μ g DNA/ml).

DNA Hydrolysis

DNA was digested to nucleosides by adapted procedures as described earlier (22,45,46). RNase-treated DNA was dissolved in Tris HCl (5 mM; pH 7.4). To 42 μ l of DNA solution (containing less than 30 μ g DNA), 21 μ l endonuclease/phosphodiesterase mix was added (consisting of 6 μ g micrococcal endonuclease/ μ l, 2.67 μ g spleen phosphodiesterase/ μ l in 60 mM sodium succinate, pH 6.0, with 30 mM CaCl₂). After incubation for 4 h at 37°C, 12.4 μ l nuclease P1 solution was added (composed of 40% vol/vol 5 μ g nuclease P1/ μ l and 60% vol/vol 0.3 mM ZnCl₂ in 0.417 M sodium acetate, pH 5.0). After incubation at 37°C for 40 min, the digestion was terminated by the addition of methanol (7.5 μ l) and HPLC eluent (83 μ l; see below), 50- μ l aliquots were directly injected into the HPLC system.

High-Performance Liquid Chromatography

The eluent (90% vol/vol citrate buffer (50 mM, pH 3.5; containing 15 μ M EDTA), and 10% vol/vol methanol) was delivered isocratically at 1.0 ml/min by a Gynkotek model 300 pump (Separations, Hendrik Ido Ambacht, The Netherlands) equipped with a membrane-type pulse dampener. Samples (50 μ l) were injected on the reverse phase column (Supelcosil LC-18-S; 250x4.6 mm, 5 μ m; Supelco, Leusden, The Netherlands) by a WISP-autosampler (Model 710B; Waters-Millipore, Bedford, MA). To quantitate normal DNA nucleosides (by UV) and 8OHdG (by ECD), column effluent was passed through an UV detector set at 254 nm (Waters, model 490; Bedford, MA) followed by an electrochemical detector (Model CU-04-AZ; Antec Leyden, Leiden, The Netherlands; potential of the glassy carbon working electrode: 700 mV vs. Ag/AgCl reference electrode). From the UV trace, amounts of 2'-deoxyguanosine (dG) were quantitated. The capillary between the UV detector and the electrochemical detector was insulated with cotton wool to reduce base line noise of the electrochemical detector. Reproducibility of the overall assay was validated with human and rat brain: multiple DNA isolations were performed with the same tissue powdered in liquid nitrogen, followed by hydrolysis and HPLC analysis.

Influence of Postmortem Delay and Storage Time on Levels of 8OHdG in Rat Brain

To study the possible influence of postmortem delay (PMD) on 8OHdG levels, brain tissue of 17 male Brown-Norway/Billingham Rijswijk (BN/BiRij) rats (age 104–126 weeks, from the inbred strain of the Gaubius Laboratory, TNO Prevention and Health) were processed after different PMDs. After cervical dislocation under mild halothane anesthesia, one hemisphere of each animal was processed after 30 min of PMD. The other hemisphere was processed at PMDs ranging from 5 to 420 min.

Effects of freezing and thawing of tissue samples on 8OHdG levels were studied with brain tissue of male BN/BiRij rats (75 weeks of age). The entire brain was homogenized in liquid nitrogen. The ratio 8OHdG/10⁵ dG in the tissue powder was determined directly, and after storage at -70°C for 1 h followed by thawing, and again after a second time of freezing and thawing. Influences of storage time on 8OHdG levels in brain tissue (1 month at -70°C) or isolated DNA (1 month at 4°C) were investigated in

triplicate using rat brain tissue of male BN/BiRij rats (75 weeks of age).

Data Presentation and Analysis

Amounts of dG and 8OHdG were quantified based on peak area measurements of the UV- and ECD-chromatograms, using the Peakmaster 3 chromatography data system (Harley Systems Ltd., Bucks, United Kingdom). The UV signals of DNA nucleosides were calibrated with dG standards. Because the dG standard contained small amounts of 8OHdG, calibrations of 8OHdG were performed in separate runs with purified 8OHdG. Oxidative damage to DNA was expressed as the ratio 8OHdG/10⁵ dG.

Results are presented as mean \pm SD. Statistically significant correlations were evaluated using multiple regression analysis (12).

RESULTS

DNA Isolation and Hydrolysis

For the determination of 8OHdG levels in human brain, DNA had to be isolated from relatively small frozen samples. The method devised in the present study allowed isolation of DNA from autopsy specimens of approximately 50 mg. Routinely, between 25 and 50 μ g of DNA was obtained, enough to complete the entire assay. Ethidium bromide/agarose gelelectrophoresis showed that the DNA was of high molecular weight, not degraded, and contained no detectable amounts of RNA.

HPLC analysis of enzymatically hydrolyzed DNA showed that the DNA was completely digested to deoxynucleosides after incubation in the micrococcal endonuclease/spleen phosphodiesterase mixture, followed by treatment with nuclease P1 (Fig. 1B).

HPLC Chromatography

Deoxynucleosides were well separated on the reverse phase column and could be conveniently detected at 254 nm. 2'-Deoxyinosine, a degradation product of 2'-deoxyadenosine, was present in all digested DNA samples (Fig. 1). By passing the effluent of the UV detector through the electrochemical detector, amounts of dG and 8OHdG were quantified within the same run (Fig. 1A and B). The identity of 8OHdG in hydrolyzed DNA was established by its retention time (identical to that of purified 8OHdG; Fig. 2), cochromatography of hydrolyzed DNA with purified 8OHdG, and by the fact that voltammograms of the purified 8OHdG standard and the putative 8OHdG peak in hydrolyzed DNA were identical ($E \approx 750$ mV; data not shown). With this analytical system, the detection limit of 8OHdG was 3 fmol per injection. Routinely, determination of 8OHdG levels in hydrolyzed DNA involved 50–200 fmol 8OHdG and 2–5 nmol dG per injection. Ratios 8OHdG/10⁵ dG as low as 0.5 could be determined reliably.

Calibration curves were linear from 20–600 fmol 8OHdG and 0.5–5 nmol dG, with correlation coefficients >0.999. Variation in the sensitivity of the HPLC assay with time was negligible: over a period of 24 h, repeated injections of the same sample resulted in variations in 8OHdG/10⁵ dG ratio of less than 5%. Reproducibility of the overall assay (DNA isolation, hydrolysis and HPLC analysis) was satisfactory: the complete procedure resulted in a coefficient of variation of 7.2% ($n = 15$). 8OHdG levels were not affected by the enzymatic hydrolysis procedure: when purified 8OHdG was subjected to the enzymatic digestion procedure (incubation with endonuclease and phosphodiesterase, followed by nuclease P1) amounts of 8OHdG determined by HPLC-ECD re-

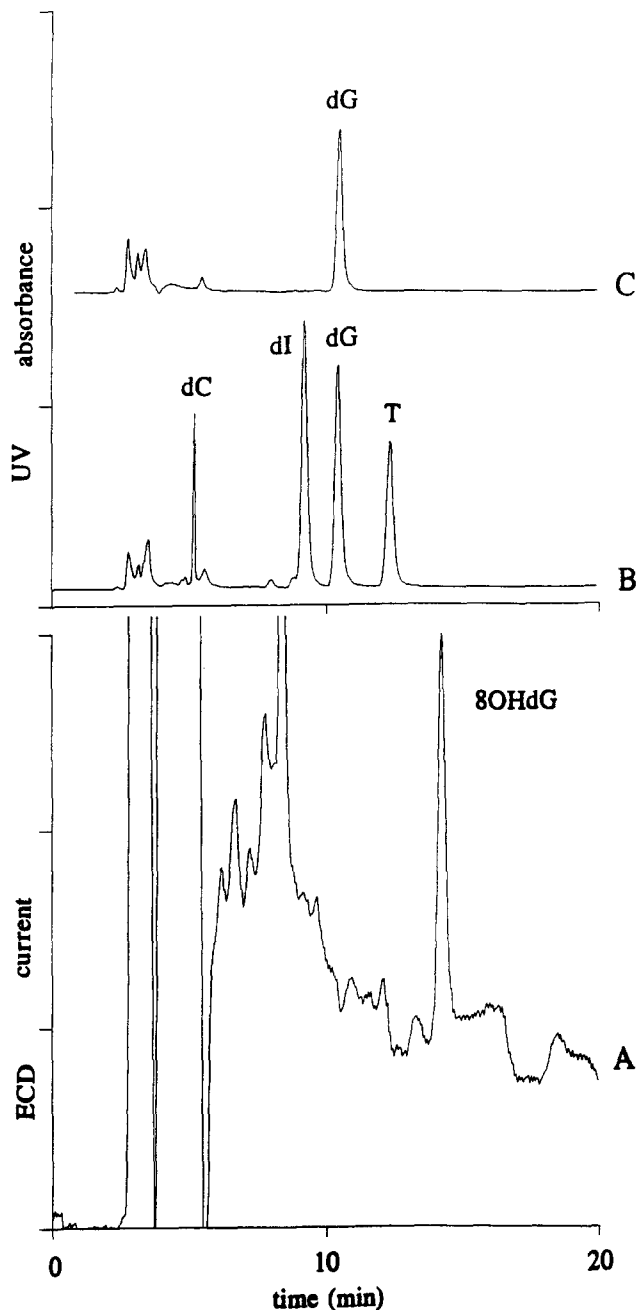


FIG. 1. HPLC chromatograms of hydrolyzed DNA, isolated from human brain tissue (control subject 91-118, frontal cortex): simultaneous UV and electrochemical detection (see the Methods section for details). Column effluent was monitored by UV-detection (trace B; 254 nm; representing 2581 pmol dG) followed by electrochemical detection (ECD; trace A; representing 141 fmol 8OHdG). Trace C; deoxyguanosine, 2000 pmol injected. dC, 2'-deoxycytosine; ECD, electrochemical detection; dG, 2'-deoxyguanosine; dI, 2'-deoxyinosine; 8OHdG, 8-hydroxy-2'-deoxyguanosine; T, thymidine; UV, ultraviolet detection at 254 nm.

mained unchanged. Furthermore, levels of 8OHdG/ 10^5 dG were independent of the amount of frozen tissue used for DNA isolation (ranging from 50 mg to 1 g), and independent of the amounts of DNA subjected to enzymatic hydrolysis and HPLC analysis (ranging from 10 to 40 μ g of DNA).

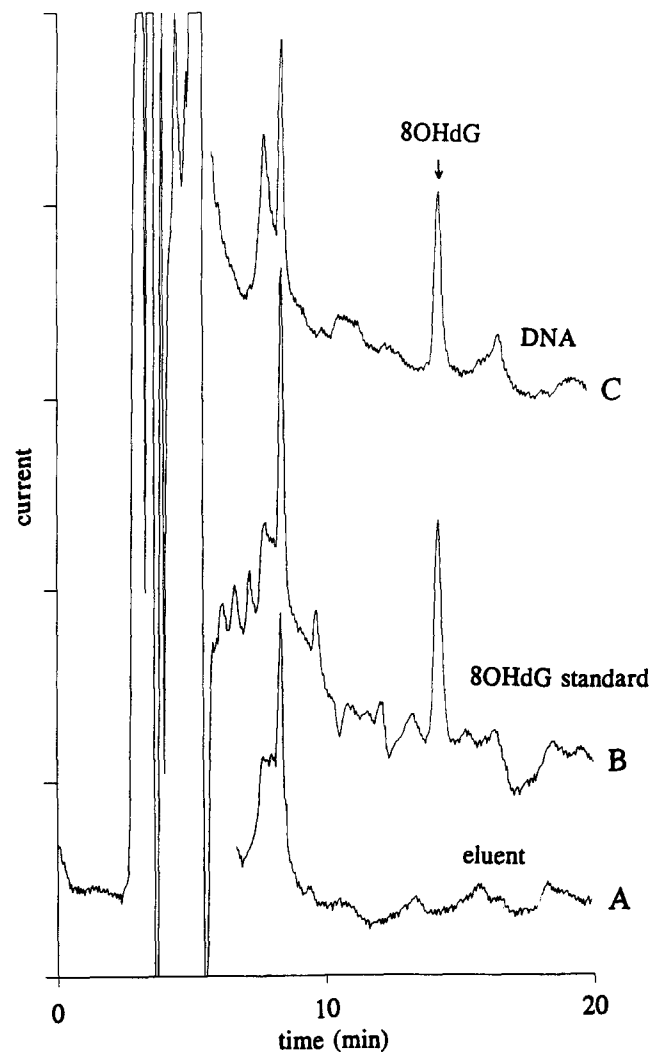


FIG. 2. HPLC chromatograms with electrochemical detection. Trace A, blank eluent; Trace B, 8OHdG standard (50 fmol injected); Trace C, hydrolyzed DNA (AD subject 92-023, frontal cortex; corresponding to a 8OHdG/ 10^5 dG ratio of 2.25).

Effect of Storage and Postmortem Delay on 8OHdG/ 10^5 dG Ratios in Rat Brain

8OHdG/ 10^5 dG ratios in directly processed frozen rat brain did not differ significantly from values obtained in brain specimens that had been thawed and frozen again once ($p > 0.2$, triplicate assays). In addition, thawing for a second time did not affect 8OHdG levels ($p > 0.5$, triplicate assays). Similarly, storage of rat brain (-70°C , 1 month) or isolated DNA (1 month at 4°C) did not influence 8OHdG levels ($p > 0.5$ and $p > 0.1$, resp.; triplicate assays).

Possible postmortem delay (PMD) influences on 8OHdG levels in brain tissue were investigated in rats with PMDs ranging from 5 min to 7 h. No statistically significant relationship of 8OHdG levels with PMD was observed (Fig. 3; $p > 0.4$).

8OHdG Levels in Postmortem Human Brain

In frontal, occipital, and temporal cortex tissue and tissue from the hippocampus, 1–7 molecules 8OHdG per 10^5 dG were mea-

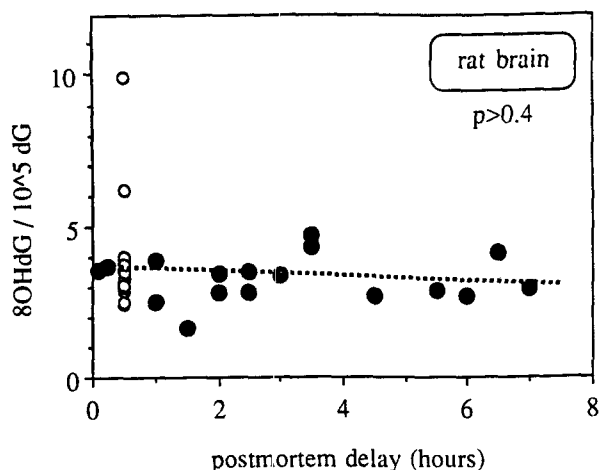


FIG. 3. Influence of postmortem delay on 8OHdG levels in rat brain. One hemisphere of each rat was processed after a postmortem delay of 30 min (open circles). The other hemisphere was processed with a postmortem delay between 5 min and 7 h (closed circles).

sured. On average, about 2 oxidized dG molecules were encountered per 10^5 dG. No age-related increases in oxidative DNA damage were observed in human brain of control subjects ($p > 0.6$; Fig. 4A; compilation of all analyzed brain areas. After omission of the high value >5 8OHdG per 10^5 dG, of subject 91-118, age 59, or 91-124, age 38; $p > 0.3$) or AD patients ($p > 0.6$; Fig. 4B; pooled data of all brain areas). In human brain, similar to rat brain, no relationship between 8OHdG levels and PMD (ranging from 2 h

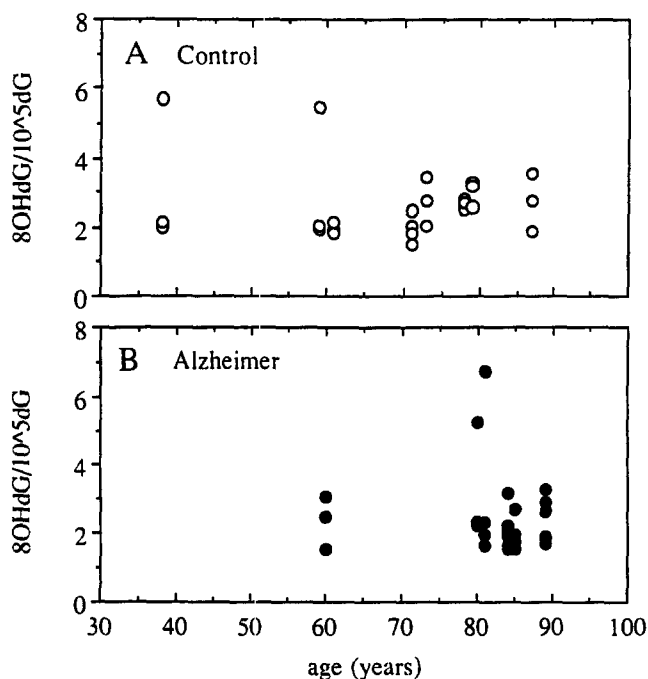


FIG. 4. Oxidative DNA damage (8OHdG/ 10^5 dG) in human brain as a function of age in control subjects (A) and Alzheimer patients (B). Data of all brain areas studied.

55 min to 8 h) was observed (Fig. 5; frontal cortex, $p > 0.2$; temporal cortex, $p > 0.05$; hippocampus, $p > 0.4$; p -values were obtained with combined data of AD and control subjects).

Levels of oxidative DNA damage were identical in different areas of the brain. No regional differences were found between frontal cortex, temporal cortex, occipital cortex, and hippocampus in control individuals ($p > 0.9$; Fig. 6A), nor in AD patients ($p > 0.8$; Fig. 6B). Levels of 8OHdG were not correlated with sex in either control individuals or AD patients ($p > 0.7$ and $p > 0.3$, respectively). Most importantly, no differences in this type of oxidative DNA damage were observed between control subjects and AD patients ($p > 0.5$; Fig. 7; compiled data of all brain areas measured). Furthermore, 8OHdG levels in specific areas of the brain (hippocampus, frontal, occipital, and temporal cortex) showed no differences between AD patients and control subjects ($p > 0.1$). Thus, the amount of 8OHdG was comparable in all areas investigated, with no differences present between AD patients and control subjects.

To substantiate that the observed levels of 8OHdG do not reflect artifacts due to experimental procedures, levels of the adduct in human brain (all subjects included in this study) were compared to 8OHdG levels in DNA isolated from WAG/Rij rats (age 53-81 weeks). The extent of oxidative DNA damage was found to be significantly higher in human brain as compared to rat brain (average levels of 8OHdG/ 10^5 dG \pm SD: human brain, 2.5 ± 1.1 , $n = 55$; rat brain, 0.55 ± 0.10 , $n = 11$; $p < 0.001$). Furthermore, a 95-fold increase in 8OHdG levels was observed when DNA was subjected to oxidative modification by vitamin C plus CuSO_4 according to others (27,39).

DISCUSSION

In the present study, existing HPLC methods were adapted to determine 8OHdG levels in small human postmortem brain samples. High molecular weight DNA, free of RNA, was obtained from small autopsy specimens of about 50 mg. By means of HPLC, amounts of dG as well as 8OHdG could be quantified reproducibly and sensitively within the same run with a detection limit for 8OHdG as low as 3 fmol per injection. Furthermore, our parametric studies show that no relationship is present between 8OHdG levels and potentially disturbing factors such as postmortem delay, repeated freezing and thawing of brain tissue, or storage of the tissue. Effects of other factors that may result in oxidative

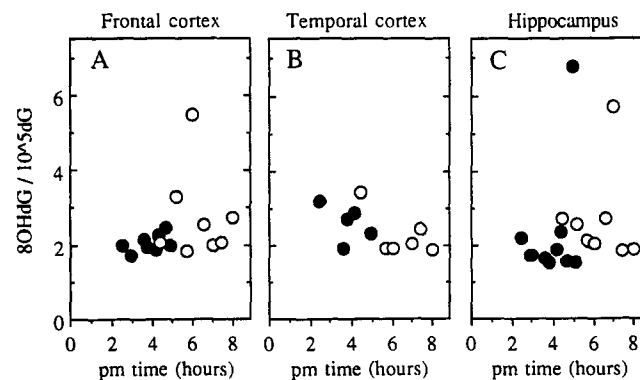


FIG. 5. Influence of postmortem delay on 8OHdG levels in human brain. Levels of oxidative DNA damage in human brain autopsy samples were plotted against the postmortem delay. (A) Frontal cortex; (B) temporal cortex; (C) hippocampus. Closed circles, AD patients; open circles, control individuals.

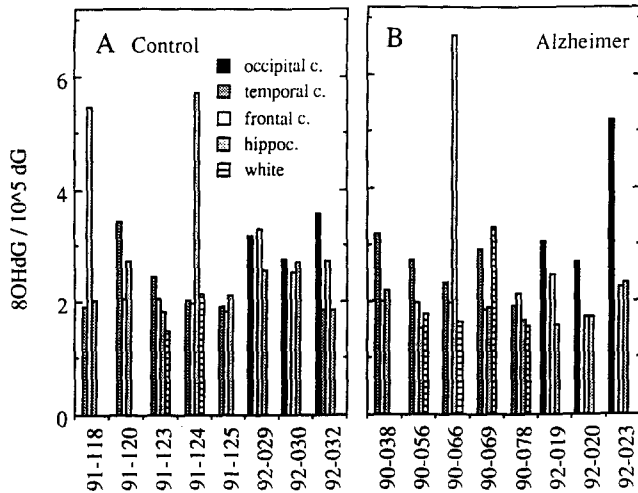


FIG. 6. 8OHdG levels in different areas of human brain of control subjects (A) and Alzheimer patients (B). Numbers indicate subject codes (see Table 1); c, cortex; hippoc., hippocampus; white, white matter. Absent bars: not determined.

stress at the time of death, such as transient brain ischemia are unknown as yet and merit further research.

As a final outcome, 8OHdG levels in human brain did not differ between the areas studied, nor were differences found between control subjects and AD patients. Approximately 2 oxidized dG molecules were present per 10⁵ dG, irrespective of the presence or

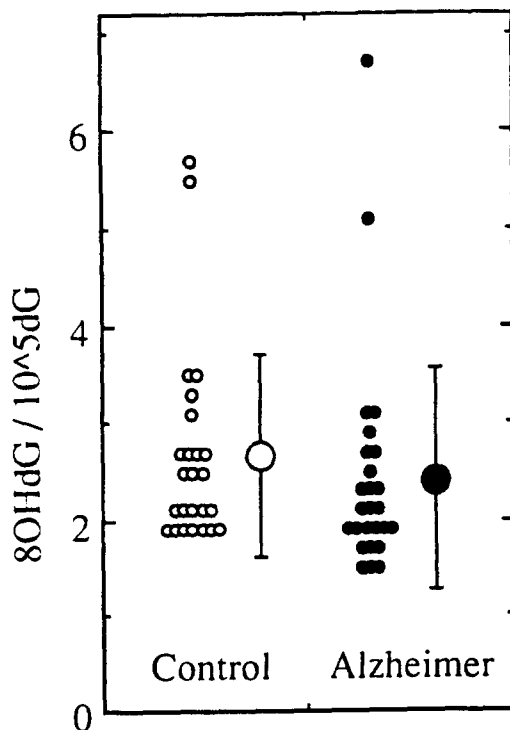


FIG. 7. Compiled data of oxidative DNA damage (8OHdG/10⁵ dG) in specimens of different brain areas (frontal, occipital and temporal cortex and hippocampus) of control subjects (25 tissue samples) and of Alzheimer patients (27 tissue samples). Comparison of control subjects and AD patients: $p > 0.5$.

absence of AD, or brain area studied. The range of 8OHdG levels (i.e., 1–7/10⁵ dG) is in good agreement with values reported in literature (22,33,34). In general, our data indicate that 8OHdG is present in the brain in fairly high levels without a preferential localization. These high basal levels of 8OHdG are in agreement with the work of others (16,33,34,39). Levels of 8OHdG have been reported to increase with age in rat liver, kidney, and intestine, but not in rat brain and testes (16), nor in human leucocytes (51). Similarly, we did not find an age-related increase in 8OHdG in human brain tissue ($p > 0.4$; $n = 55$, pooled data of control subjects, Fig. 4A, and Alzheimer patients, Fig. 4B). At first sight, our data seem to deviate from those reported by Mecocci et al. (33,34): they showed a weak correlation of 8OHdG levels in nuclear DNA of control subjects with age ($r = 0.47$, $p = 0.03$; all analyzed samples taken together). In particular, two subjects aged over 90 years contributed to this conclusion. Furthermore, 8OHdG levels were only correlated with age in cerebellum, and not in frontal, parietal or temporal cortex (34). Our data of control subjects, covering age 38–87 without individuals with age >90 (Fig. 4A), are consistent with those of Mecocci et al. (34). However, statistical analysis of our data does not allow the conclusion that levels of 8OHdG in nuclear DNA increase with age ($p > 0.6$).

Furthermore, our data do not support differences in 8OHdG levels in nuclear DNA between AD patients and control subjects, in contrast to Mecocci et al. (33). Here again, both data sets are virtually identical, but lead to different conclusions. In both studies, 8OHdG levels in specific brain areas are identical between AD and control. Furthermore, in both studies, 8OHdG levels in AD and control are identical when all tissues analyzed are combined, and when expressing oxidative damage as the 8OHdG/10⁵ dG ratio. Surprisingly, a significant difference between AD and control was only observed if oxidative DNA damage of the pooled data was expressed as fmol 8OHdG/ μ g DNA (33). Our study was restricted to oxidative DNA damage expressed as the ratio 8OHdG/10⁵ dG. Altogether, both data sets are essentially not different, but lead to different conclusions.

The hypothesis that oxidative DNA damage is involved in the etiology of AD suggests that increased levels of 8OHdG should be most easily detected in temporal cortex and hippocampus, the brain areas most affected by AD. However, our data (Fig. 5B) and those of Mecocci et al. (33) show that differences between AD and control are lacking in temporal cortex. Similarly, levels of 8OHdG in hippocampus were not different between AD and control (Fig. 5C). Thus, comparison of oxidative DNA damage in different areas of the brain does not support a role of oxidative DNA damage in the etiology of AD.

A shortcoming of the present data set is the difference in PMD and age of AD patients and control subjects (Table 1). However, both factors do not confound our conclusions for 1) PMD (4:00 \pm 0.50 h in AD; 6:17 \pm 1.12 h in control) showed no relationship with 8OHdG levels (Fig. 5); and 2) the rat study designed to evaluate the effect of PMD on 8OHdG levels also does not support a relationship between PMD and 8OHdG levels (Fig. 3). Similarly, the shortcoming that age of control (68.2 \pm 15.3) and AD (83.8 \pm 7.3) groups are different does not confound our conclusion since 1) we show that 8OHdG levels in nuclear DNA do not increase with age; and 2) if age-related accumulation of 8OHdG was to occur (34), our significantly older AD group would have had higher 8OHdG levels than the younger control group. However, this is not what we found: 8OHdG levels in AD and control subjects did not differ from each other.

Whereas the present study, altogether, does not support a role for oxidative DNA damage in the etiology of AD, it does not take into account local differences in nuclear 8OHdG levels between particular brain areas (on a microscopic level), or between particu-

lar cell types (e.g., glia vs. neurons), or loss of oxidative DNA damage due to cell death. Such effects cannot be detected with the present HPLC method where brain homogenates have been used. Investigations to address these issues require a (immuno)histological approach that is presently not available.

In addition, besides damage to nuclear DNA, mitochondrial DNA which accounts for about 1% of the total cellular DNA has been suggested as a target for oxidative damage (3,8,30,37,48,53). 8OHdG levels in mitochondrial DNA have been shown to be tenfold higher than those in nuclear DNA and show prominent increases in both normal aging and AD (33,34,39). It is clear that on the basis of 8OHdG levels, mitochondrial DNA is much more affected than nuclear DNA in both conditions (33,34). Whether increased levels of 8OHdG in mitochondrial DNA play a causal role in AD etiology or are merely a consequence of the disease, merits further investigations.

We conclude that previous studies (33,34) as well as the present data demonstrate relatively high basal levels of 8OHdG throughout

life, without a preferential anatomical localization. As far as damage to nuclear DNA in brain is concerned, our present findings on 8OHdG do not demonstrate an accumulation of this particular oxidative DNA adduct with age. Other types of oxidative DNA damage, however, may be involved in AD etiology.

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