Effect of Oxidative Stress on DNA Damage and β-Amyloid Precursor Proteins in Lymphoblastoid Cell Lines from a Nigerian Population

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INTRODUCTION

Neurodegenerative changes are a characteristic feature of the aging process. These changes may result from damage to intracellular macromolecules by reactive oxygen species (ROS) generated during oxidative phosphorylation and other enzymatic reactions.[1] The ability to repair macromolecular damage also decreases with age.[2] As one of the most metabolically active organs, the brain is considered to be most vulnerable to oxidative stress. Age is a well-established risk factor for late-onset sporadic Alzheimer's disease (AD), and the extent of neurodegenerative changes is significantly greater in AD patients than in controls. There are alterations in several markers of oxidative stress in brain tissue from AD patients, suggesting a link between increased oxidative stress and cell damage.[1] Since mitochondria are a major source of ROS, it has been hypothesized that increased cellular damage in AD may be the result of a genetic defect that occurred in a somatic cell at an early stage of development.[3] Such a defect may promote mitochondrial DNA damage or impair DNA repair.[4] Somatic mutations in mitochondrial (or nuclear) genes encoding enzymes of energy metabolism may also explain the pathological changes.[5] AD is primarily a neuronal disease, but the occurrence of a somatic mutation in early embryogenesis could explain the systemic features of this disorder.[3,6] It is on the basis of observations like these that ROS has been implicated in the pathogenesis of AD. Brain autopsy studies have shown that the density of plaques is lower in elderly Nigerians compared with Caucasian populations.[7] The age-adjusted prevalence of AD in an elderly population group in Ibadan (Nigeria) was significantly lower compared with an African-American population group in Indianapolis (Indiana).[8] The ε4 allele of apolipoprotein E (APOE) is a major risk factor for the development of late-

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onset sporadic AD in Caucasian and Japanese populations.[9] Our studies have shown that APOE ε4 was a weak risk factor for AD in the Indianapolis group, but it was not a risk factor in the Ibadan group.[10] The conversion of the soluble amyloid-β (Aβ) peptides into the insoluble amyloid present in neuritic plaques is accelerated in the presence of APOE ε4,[11] and free radical reactions are involved in this process.[12] These observations suggest that the lower density of plaques in brains from Nigerian subjects may be related to reduced oxidative stress, but this remains to be established in AD patients.

Because of the problems associated with obtaining brain tissue, molecular analyses on AD have been carried out in lymphoblast or fibroblast cell lines.[13] The use of non-neuronal tissue is also justified in view of the alterations that have been demonstrated in fibroblast and other cell types from AD patients.[6] We have established transformed lymphoblast cell lines from several probable AD cases and non-demented controls from Indianapolis and Ibadan. The APOE genotypes of these individuals are known. In this preliminary study, we investigated the effects of endogenous and induced oxidative stress on total (nuclear and mitochondrial) DNA damage in eight cell lines (five probable AD and three controls) from the Ibadan group. The effect of oxidative stress on the level of a putative protein marker for AD (β-amyloid precursor protein, APP) was also examined.[14] The level of the heat shock protein (HSP-70) was used as a control.

EXPERIMENTAL PROCEDURES

Materials

Acrylamide, alkaline phosphatase, nuclease P1, sodium dodecyl sulfate (SDS), *t*-butyl peroxide and other chemicals were obtained from Sigma (St. Louis, MO). The growth medium, serum and other cell culture reagents were procured from Life Technologies (Gaithersburg, MD).

Cell Culture

Cells were grown in RPMI 1640 containing 7.5% fetal calf serum. For each cell line, 10 ml aliquots (about 7×10^6 cells) were used in triplicate: one each for total DNA (nuclear and mitochondrial) isolation, nuclear DNA isolation, and protein expression studies. This was performed before and after exposing the cells to 200 μM *t*-butyl peroxide for 4 hours. This chemical generates hydrogen peroxide, which is known to damage DNA through free radical formation.

Oxidative DNA Damage

Total DNA was isolated from untreated and treated samples by extracting with phenol-chloroform. For nuclear DNA, nuclei were first isolated by gentle homogenization. The DNA was digested with nuclease P1 and alkaline phosphatase and then analyzed by HPLC for 8-hydroxy-2′-deoxyguanosine (OH8dG), a marker for oxidative DNA damage. The level of oxidative DNA damage was expressed as μmol OH8dG per mol dG. Mitochondrial DNA damage was taken to be the difference between the total and nuclear DNA.

TABLE 1. Diagnosis, gender and APOE genotyping of different subjects

Subject/Cell line	Gender	Age	Diagnosis	APOE
1586	F	88	NDEM	3/3
1588	F	81	DEM	4/4
1589	F	115	DEM	3/3
1590	F	88	DEM	2/3
1593	F	73	CI	3/3
1597	F	85	DEM	3/4
2198	M	97	DEM	3/3
2205	M	70	NDEM	3/3

NOTES: Cell lines, derived from these subjects, were used in subseqent studies (TABLES 2 and 3). Clinical diagnosis was based on DSM-III and NINDS/ADRDA criteria (see Hendrie *et al*., 1995). NDEM: non-demented; DEM: demented; CI: cognitively impaired.

TABLE 2. Levels of 8-hydroxy-2′-deoxyguanosine (OH8dG) and 2′-deoxyguanosine (dG), and the ratio of OH8dG/dG, in untreated and treated cell lines

	Untreated			Treated		
Subject/Cell Line	OH8dG fmol	dG pmol	Ratio $\times 10^{-6}$	OH8dG fmol	dG pmol	Ratio $\times 10^{-6}$
1586	9.12	1007.07	9.06	9.03	439.29	20.56
1588	8.30	2032.81	4.08	16.95	1024.32	16.55
1589	15.93	2037.93	7.82	5.79	1024.75	5.65
1590	6.26	1794.72	3.49	7.18	1796.34	4.00
1593	13.94	1166.60	11.95	9.79	842.85	11.62
1597	9.73	767.37	12.68	9.03	1221.66	7.39
2198	13.96	1633.54	8.55	10.38	289.83	35.81
2205	5.09	454.59	11.20	20.27	1599.69	12.67

NOTES: DNA was digested with nuclease P1 and alkaline phosphatase and analyzed by HPLC. Levels of OH8dG were determined by electrochemical detection and dG by UV detection. The level of oxidative damage was expressed as the OH8dG/dG ratio.

Protein Levels

The untreated and treated samples were centrifuged and the conditioned medium saved. The cell pellets were re-suspended in buffer containing detergents and protease inhibitors. The cell suspension was sonicated, and the protein concentration determined. Thirty μg protein from the cell lysate was separated on a 10% polyacrylamide gel containing SDS. Immunodetection of specific bands was performed as previously described.[14] For the detection of APP and its derivatives, primary antibody mAb22C11 (Roche Molecular Biochemicals, Indianapolis, IN) was used. This antibody recognizes all mature forms of APP found in cell membranes, the carboxyl-truncated soluble forms secreted into the medium, and the APP-like protein (APLP). The 6E10 monoclonal antibody, which recognizes residues 1–28 of the APP-specific extra-membranous region of the Aβ sequence, was also used to confirm the APP band (data not shown). Primary antibody N27F3-4 (Sigma) was used for the detection of HSP-70/72, which served as an internal control.

TABLE 3. Qualitative changes in the OH8dG/dG ratio and the expression of APP and HSP following treatment of cell lines with *tert*-butyl peroxide

Subject/ Cell Line	Diagnosis	OH8dG/dG Ratio	APP	HSP
1586	NDEM	↑	–	↓
1588	DEM	↑	↓	↓
1589	DEM	–	–	–
1590	DEM	–	↓	–
1593	CI	–	–	–
1597	DEM	↓	↓	↓
2198	DEM	↑	↓	↓
2205	NDEM	–	↓	–

NOTES: The ratio of OH8dG/dG and the level of APP, HSP proteins are increased (↑), decreased (↓), or unchanged (–) in the treated cell lines relative to controls.

RESULTS

The clinical status and APOE genotypes of the patients; the levels of OH8dG, dG, and the OH8dG/dG ratio; and qualitative changes in APP and HSP-70/72 expression are shown in TABLES 1–3. The levels of APP and HSP-70/72, before and after *t*-butyl peroxide treatment, were measured semiquantitatively from Western blots. These changes are summarized in TABLE 3. In this preliminary study, we did not detect a significant difference in the OH8dG/dG ratio in total DNA in cell lines from patients or controls, with or without treatment with *t*-butyl peroxide. The ratio for the untreated group was in the range 3.8 to 13.2, and similar values were obtained in the treated group (range 3.9 to 37.1). There was also little or no change in APP and HSP-70/72 expression following oxidative stress. Our sample size was too small to make any correlation between these changes and APOE genotypes.

We also carried out oxidative stress studies in lymphoblastoid cell lines from two autopsy-confirmed cases of AD, six cases of probable AD, and three controls (all of Caucasian origin). We observed a 2- to 3-fold increase in OH8dG formation in total DNA, but not nuclear DNA, following *t*-butyl peroxide treatment in the patient group, but not in the control group (Sahota *et al*., unpublished data). This suggested that there was increased mitochondrial damage in cell lines from AD patients compared with controls following oxidative stress.

DISCUSSION

ROS have been implicated in the pathogenesis of AD and other neurodegenerative diseases.[15] Although alterations in several markers of oxidative stress have been reported in AD patients, a cause and effect relationship between ROS and AD remains to be established. Impaired energy metabolism,[16] mutations in genes encoding enzymes of oxidative phosphorylation,[5] and increased mitochondrial DNA

damage and/or defective repair strongly suggest a role for oxidative stress in the pathogenesis of AD.[4]

Our study of dementia in two populations of African origin suggested that the prevalence of AD was significantly lower in Nigerian blacks compared with African-Americans.[8] Non-demented elderly subjects from Nigeria also had a lower density of senile plaques compared with Caucasian subjects.[7] We have shown that APOE ε4, the gene product of which has been shown to promote free radical formation,[12] is not associated with AD in Nigerian blacks.[10] The lower density of plaques in brain tissue and the lower prevalence of AD may be related to reduced oxidative stress in this population.

In this preliminary study, we did not detect a difference in endogenous or induced DNA damage in cell lines from patients or controls from Ibadan, and this effect was independent of APOE genotype. We have observed an increase in mitochondrial DNA damage in cell lines from a small number of probable AD patients of Caucasian origin (Sahota *et al*., unpublished data). Based on the cell culture studies, the absence of oxidative DNA damage in the Ibadan subjects may be related to the lower prevalence of AD in this population. Our findings also indicate that APP and its derivatives are expressed not only in neuronal cells but also in lymphoblastoid cell lines. Lymphoblast cell lines may prove to be useful tools for evaluating oxidative stress status, mitochondrial mutations, AD protein markers, and the risk for AD in epidemiological and clinical studies.

SUMMARY

The ε4 allele of apolipoprotein E (APOE) is strongly associated with late-onset Alzheimer's disease (AD) in Caucasian populations, but our studies suggest that APOE ε4 is not a risk factor for AD in Nigerian blacks and is a weak risk factor in African-Americans. The prevalence of AD is lower in Nigerians than in African-Americans. Increased oxidative damage to macromolecules in brain tissue by reactive oxygen species (ROS) has been reported in AD. Here we examined the effects of endogenous and induced oxidative stress on total (nuclear and mitochondrial) DNA damage in lymphoblastoid cell lines (5 probable AD and 3 controls) from Ibadan, Nigeria. Cells were exposed to 200 μM *t*-butyl peroxide (a generator of ROS) for 4 hours. Total DNA was isolated and digested with nuclease P1 and alkaline phosphatase. DNA fragments were separated by HPLC and the levels of 8-hydroxy-2′-deoxyguanosine (OH8dG, an indicator of DNA damage) and deoxyguanosine (dG) determined. We did not detect a significant difference in the OH8dG/dG ratio in untreated or treated cell lines in the two groups, and this was independent of APOE genotype. We also examined, by Western blotting, the level of β-amyloid precursor protein (APP) which is involved in AD. The level of the heat shock protein (HSP-70) was examined as a control. There was a slight decrease in levels of APP and HSP-70 following treatment. Studies in cell lines from Caucasian subjects have shown an increase in mitochondrial DNA damage following oxidative challenge. Our preliminary results suggest that African populations are less vulnerable to chemical-induced oxidative DNA damage.

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