Free Radical Biology & Medicine 45 (2008) 813–819

Contents lists available at ScienceDirect

Free Radical Biology & Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Altered 8-oxoguanine glycosylase in mild cognitive impairment and late-stage Alzheimer's disease brain

Changxing Shao [a], Shuling Xiong [b], Guo-Min Li [c,d], Liya Gu [c,d], Guogen Mao [c], William R. Markesbery [b,e], Mark A. Lovell [a,b,*]

[a] Department of Chemistry, University of Kentucky, Lexington, KY 40506, USA
[b] Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40536, USA
[c] Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40536, USA
[d] Department of Pathology & Laboratory Medicine, University of Kentucky, Lexington, KY 40536, USA
[e] Departments of Pathology and Neurology, University of Kentucky, Lexington, KY 40536, USA

ARTICLE INFO

Article history:
Received 21 February 2008
Revised 5 May 2008
Accepted 3 June 2008
Available online 11 June 2008

Keywords:
Oxoguanine glycosylase
Lipid peroxidation
Alzheimer's disease
Mild cognitive impairment
4-Hydroxynonenal

ABSTRACT

Eight-hydroxy-2′-deoxyguanosine (8-OHdG) is increased in the brain in late-stage Alzheimer's disease (LAD) and mild cognitive impairment (MCI). To determine if decreased base-excision repair contributes to these elevations, we measured oxoguanine glycosylase 1 (OGG1) protein and incision activities in nuclear and mitochondrial fractions from frontal (FL), temporal (TL), and parietal (PL) lobes from 8 MCI and 7 LAD patients, and 6 age-matched normal control (NC) subjects. OGG1 activity was significantly ($P<0.05$) decreased in nuclear specimens of FL, TL, and PL in MCI and LAD and in mitochondria from LAD FL and TL and MCI TL. Nuclear OGG1 protein was significantly decreased in LAD FL and MCI and LAD PL. No differences in mitochondrial OGG1 protein levels were found. Overall, our results suggest that decreased OGG1 activity occurs early in the progression of AD, possibly mediated by 4-hydroxynonenal inactivation and may contribute to elevated 8-OHdG in the brain in MCI and LAD.

Introduction

Increasing evidence supports a role for oxidative damage in the pathogenesis of a variety of neurodegenerative diseases including Alzheimer's disease (AD). Reactive oxygen species (ROS) may be particularly damaging to brain because of its lipid-rich environment and relatively low antioxidant capacity. ROS may interact with and damage lipids, proteins, RNA, and DNA (reviewed in [1]). Studies show significantly elevated levels of oxidative damage to lipids including formation of aldehydic by-products 4-hydroxynonenal (HNE) and acrolein in vulnerable regions of the brain in late-stage AD (LAD) [2–4] and in mild cognitive impairment (MCI) [5–7], the earliest clinical manifestation of AD. In addition, several studies show significant elevations of markers of oxidation in nuclear and mitochondrial DNA isolated from LAD (reviewed in [8]) and MCI [9] subjects and in RNA from LAD [10–14] and MCI [15] as well as other neurologic disorders including Parkinson's disease [16] and diffuse Lewy body disease (DLB) [17]. Because mitochondria generate most free radicals, their DNA are particularly susceptible to oxidative damage. Comparison of nuclear and mitochondrial DNA oxidative damage shows that mitochondrial levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) are significantly higher than those observed in nuclear DNA in MCI [9] and LAD [18,19].

Although several oxidized bases can result from attack of DNA by ROS, the predominant marker is 8-OHdG [20]. 8-OHdG is potentially mutagenic and, if unrepaired, improperly pairs with adenine during replication and induces G:C → A:T transversion mutations [21]. In postmitotic neurons, accumulation of oxidatively modified DNA bases may result in diminished cellular activity and death [22]. Protection against accumulation of 8-OHdG in human cells is mediated in part by the base-excision repair (BER) pathway that is initiated by excision of oxidized guanine by 8-oxoguanine DNA glycosylase (OGG1).

There are few studies of OGG1 and its activity in the progression of AD. Initial studies from our laboratory showed decreased OGG1 activity in nuclear fractions from vulnerable regions of LAD brain compared to age-matched normal control (NC) subjects [23]. Using immunohistochemistry, Mao et al. [24] showed *OGG1* mutations that led to significant decreases in OGG1 activity in 4 of 14 LAD subjects

Abbreviations: AD, Alzheimer's disease; BER, base-excision repair; CER, cerebellum; DTT, dithiothreitol; FL, frontal lobe; HNE, 4-hydroxynonenal; LAD, late-stage Alzheimer's disease; MCI, mild cognitive impairment; NC, normal control; NFT, neurofibrillary tangle; PL, parietal lobe; PMI, postmortem interval; OGG1, oxoguanine glycosylase 1; 8-OHdG, eight-hydroxy-2′-deoxyguanosine; PBS, phosphate-buffered saline; ROS, reactive oxygen species; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TL, temporal lobe.

* Corresponding author. 135 Sanders-Brown Center on Aging, University of Kentucky, 800 S. Limestone St., Lexington, KY 40536-0230, USA. Fax: +1 859 323 2866.
E-mail address: malove2@uky.edu (M.A. Lovell).

0891-5849/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.freeradbiomed.2008.06.003

analyzed. In contrast, studies of OGG1 in MCI have been limited. Recent studies of Weissman et al. [25] showed no significant change in OGG1 excision activity in MCI IPL compared to NC subjects. No studies of OGG1 excision activities in mitochondrial fractions from MCI or LAD brain have been reported.

To determine if elevations of 8-OHdG in mitochondrial and nuclear DNA from vulnerable regions of MCI and LAD brain are due to altered OGG1 activity, we determined OGG1 protein levels and 8-OHdG incision activities in nuclear and mitochondrial enriched fractions from frontal (FL), parietal (PL), and temporal (TL) lobe and cerebellum (CER) from MCI, LAD, and age-matched NC.

Materials and methods

Subject selection and neuropathologic examination

To isolate sufficient amounts of mitochondria for activity analyses relatively large brain specimens (~2 g) were obtained from frontal, parietal, and temporal lobes and CER from short postmortem interval (PMI) autopsies of 7 (4 men, 3 women) LAD patients, 8 (2 men, 6 women) MCI patients, and 6 (4 men, 2 women) age-matched NC subjects through the Neuropathology Core of the University of Kentucky Alzheimer's Disease Center (UK-ADC). These brain regions were chosen for analysis to allow comparison of OGG1 activity and protein to levels of 8-OHG measured in previous studies [9,19]. All LAD, MCI, and NC subjects were followed longitudinally in the Clinical Core of the UK-ADC and had annual neuropsychological testing and physical and neurological examinations. LAD patients demonstrated progressive cognitive decline and met NINDS/ADRDA accepted standard criteria for the clinical diagnosis of probable AD [26]. All NC subjects had neuropsychologic scores in the normal range and showed no evidence of memory decline. Subjects with amnestic MCI were derived from the control group and were normal on enrollment into the longitudinal study and developed MCI during follow-up. The clinical criteria for diagnosis of amnestic MCI are those of Petersen et al. (1999) and include: (a) memory complaints, (b) objective memory impairment for age and education, (c) intact general cognitive function, (d) intact activities of daily living (ADLs), and (e) the subject is not demented. Objective memory test impairment was based on a score of ≤1.5 standard deviations from the mean of controls on the CERAD Word List Learning Task [27] and corroborated in some cases with the Free and Cued Selective Reminding Test.

All subjects had neuropathological examination of multiple sections of neocortical association areas, hippocampus, entorhinal cortex, amygdala, basal ganglia, nucleus basalis of Meynert, midbrain, pons, medulla, and cerebellum using hematoxylin and eosin stain, the modified Bielschowsky stain, and Aβ and α-synuclein immunostains. Braak staging [28] scores were determined using the Gallyas stain on sections of entorhinal cortex, hippocampus, and amygdala and the Bielschowsky stain on neocortical sections. All LAD patients met accepted criteria for the histopathologic diagnosis of AD (high likelihood NIA-Reagan Institute [NIA-RI] criteria) [27,29,30] and typically demonstrated Braak staging scores of VI.

Histopathologic examination of NC subjects generally showed only age-associated changes and Braak staging scores of I to III and met NIA-RI low likelihood criteria for the histopathologic diagnosis of AD. The major distinction between MCI and NC subjects was a significant increase in neuritic plaques in neocortical regions and a significant increase in NFT in entorhinal cortex, hippocampus, and amygdala in MCI compared to NC subjects [31]. Braak staging scores of MCI subjects typically ranged from III to IV.

Preparation of nuclear and mitochondrial enriched fractions

Enriched nuclear and mitochondrial fractions were prepared using the method of Mecocci et al. [32] with modification [19]. Briefly, brain specimens were homogenized on ice using a motor-driven Teflon-coated Dounce homogenizer in MSB-Ca^{2+} buffer (0.21 M mannitol, 0.07 M sucrose, 0.05 M Tris-HCl, 3 mM $CaCl_2$, pH 7.5). Disodium EDTA (0.01 M) was added to the homogenate, which was centrifuged at 1500 g and 4°C for 20 min. The pellet was resuspended in MSB-Ca^{2+} buffer and centrifuged again to yield the nuclear fraction. The combined supernatant was centrifuged at 20,000 g and 4°C for 20 min to yield a crude mitochondrial pellet. The pellet was washed with MSB-Ca^{2+} buffer and resuspended in 2 ml MSB-Ca^{2+}, loaded onto a 1:1 Percoll/ MSB-Ca^{2+} gradient, and centrifuged at 50,000 g and 4°C for 1 h. Mitochondria were isolated at a density of 1.035 g/ml. The mitochondria were resuspended in MSB-Ca^{2+} buffer and centrifuged through a second Percoll gradient leading to purified intact mitochondria. The mitochondrial pellet was resuspended in 300 μl 20 mM Hepes-KOH (pH 7.6) containing 1 mM EDTA, 2 mM DTT, 300 μM KCl, 5% glycerol, and 0.05% Triton-X (MSHE buffer). Purity of isolated fractions was verified using Western blot analysis of representative mitochondrial and nuclear specimens and antibodies against lamin A, a nuclear specific marker and porin, a mitochondria specific marker.

8-Oxoguanine glycosylase activity assay

8-Oxoguanine glycosylase activity assays were carried out as previously described [33] using an oligonucleotide duplex consisting of ^{32}P-labeled 5′-GAACGACTGT(oxoG)ACTTGACTGCTACTGAT-3′ and unlabeled complementary strand (5′-ATCAGTAGCAGTCAAGT-CACAGTCGTTC-3′) (Midland Certified Reagent Company). For OGG1 reactions, a 20 μl reaction mixture containing 40 mM Hepes-KOH (pH 7.6), 5 mM EDTA, 2 mM DTT, 75 mM KCl, 10% glycerol and 88.7 fmol ^{32}P-labeled duplex oligonucleotide was mixed with 20 μg nuclear or mitochondrial protein and incubated at 37°C for 1 h. Reactions were quenched by adding an equal volume of loading buffer containing 90% formamide, 0.002% bromophenol blue, and 0.002% xylene green and heating at 95°C for 5 min. The reaction mixture was cooled to room temperature and separated on a 20% polyacrylamide gel containing 7 M urea prerun at 18 W for 30 min. The samples were separated by electrophoresis at 16 W for 90 min using 1X Tris borate EDTA (TBE) (pH 8.0) as a running buffer. Blanks for the assay consisted of reaction mixture and 20 μg protein specimens that were heat-inactivated by boiling at 95°C for 5 min before initiating the activity assay. Samples were analyzed with NC and MCI or NC and LAD samples on the same gel to allow comparison. Radiolabeled bands were visualized using a Fuji FLA-2000 phosphorimager after exposure of the imaging plate for 24 h. Band intensities of the cleaved product and parent oligonucleotide bands in each lane were quantified using Image-Quant software (Molecular Dynamics). Results of the assay are reported as the mean±SEM amount of radioactivity in the incised product band relative to the total radioactivity in the lane (incised product + intact duplex oligonucleotide).

Single-strand conformational polymorphism analysis

To determine possible mutations in the *OGG1* gene, PCR-based single-strand conformational polymorphism was used as previously described [24] with primers against all seven exons and flanking splice sites of the *OGG1* gene.

Western blot analyses for OGG1

Specimens (25 μg) of nuclear or mitochondrial fractions were subjected to SDS-PAGE and transferred to nitrocellulose. Gels were loaded with NC and MCI or NC and LAD specimens by brain region to allow comparison. The Western blots were blocked for 2 h in 5% dry milk/Tris buffered saline containing 0.1% Tween 20 (TTBS) followed by incubation overnight in a 1:100 dilution of rabbit anti-human OGG1