Neurobiology of Aging 35 (2014) 1293–1300

Contents lists available at ScienceDirect

Neurobiology of Aging

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

Base excision DNA repair levels in mitochondrial lysates of Alzheimer's disease

Chandrika Canugovi, Raghavendra A. Shamanna, Deborah L. Croteau, Vilhelm A. Bohr*

Laboratory of Molecular Gerontology, National Institute on Aging, Baltimore, MD, USA

ARTICLE INFO

Article history:
Received 21 September 2013
Received in revised form 20 December 2013
Accepted 4 January 2014
Available online 8 January 2014

Keywords:
Base excision repair
5-Hydroxyuracil
Ligation
Alzheimer's disease
Mitochondria

ABSTRACT

Alzheimer's disease (AD) is a senile dementia with increased incidence in older subjects (age >65 years). One of the earliest markers of AD is oxidative DNA damage. Recently, it has been reported that preclinical AD patient brains show elevated levels of oxidative damage in both nuclear and mitochondrial nucleic acids. Moreover, different oxidative lesions in mitochondrial DNA are between 5- and 10-fold higher than in nuclear DNA in both control and AD postmortem brains. We previously showed that there is a significant loss of base excision repair (BER) components in whole tissue extracts of AD and mild cognitive impairment subjects relative to matched control subjects. However, comprehensive analysis of specific steps in BER levels in mitochondrial extracts of AD patient brains is not available. In this study, we mainly investigated various components of BER in mitochondrial extracts of AD and matched control postmortem brain samples. We found that the 5-hydroxyuracil incision and ligase activities are significantly lower in AD brains, whereas the uracil incision, abasic site cleavage, and deoxyribonucleotide triphosphate incorporation activities are normal in these samples.

Published by Elsevier Inc.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder leading to dementia, a common term used to describe memory and cognitive impairment (Perez et al., 1975). At the molecular level, AD pathology is marked by the extracellular amyloid-beta plaque formation and/or intracellular tau tangle formation (Ikeda et al., 1989; Masters and Beyreuther, 1995). Previous reports have also associated mitochondrial dysfunction with AD (Liang et al., 2008; Trimmer et al., 2000). A consistent defect in AD has been a deficiency in cytochrome c oxidase, an enzyme essential for oxidative phosphorylation (OXPHOS), which was initially reported in AD platelets (Bosetti et al., 2002) and later in postmortem brain tissue from patients with AD (Maurer et al., 2000). Loss of mitochondrial genomic integrity is also implicated in AD pathology (Coskun et al., 2012). The mitochondrial inner membrane anchors mitochondrial DNA (mtDNA) which is the site for OXPHOS that generates adenosine triphosphate (ATP); however, reactive oxygen species (ROS) are also produced as a byproduct. The close proximity of mtDNA to the ROS source makes mtDNA more vulnerable to oxidative damage, and damaged mtDNA, which codes for OXPHOS protein components, can cause mitochondrial dysfunction (Onyango et al., 2006; Yang et al., 2008). mtDNA deletions and elevated levels of specific oxidative lesions have been reported to be present in postmortem brain samples (Aliyev et al., 2005; Krishnan et al., 2012; Melberg et al., 2005; Phillips et al., 2013; Wang et al., 2005, 2006). Additionally, degraded mtDNA and proteins were found in the secondary lysozomes, and swollen mitochondria with damaged cristae are found in the neurons of AD brains (Baloyannis, 2006; Baloyannis et al., 2004; Reddy, 2009). Together, these data and many others (Coskun et al., 2012) indicate that mitochondrial dysfunction and genomic instability may be the main contributors to AD pathology.

Neither the importance of base excision repair (BER) proteins in normal brain function, including memory retention and cognition, nor the relative importance of nuclear versus mtDNA BER repair is well understood at this time. Notably, DNA repair processes in mitochondria are not as comprehensive as they are in the nucleus (Croteau et al., 1999), but BER is well conserved in this compartment (Bohr and Dianov, 1999; Dianov et al., 2001). Previously, we showed that the BER pathway is defective in AD postmortem brain whole tissue lysates (Weissman et al., 2007); however, it is not known whether there are deficits in specific steps of BER in mitochondria of AD brain samples. BER consists of 4 major steps. DNA glycosylases recognize and remove specific damaged bases leaving an abasic site. Apurinic/apyrimidinic (AP) endonucleases process abasic sites generating a single-stranded gap in the DNA. The gap is filled in by a DNA polymerase, and then ligase seals the nick to complete the DNA repair process

* Corresponding author at: Laboratory of Molecular Gerontology, National Institute on Aging, 251 Bayview Blvd, Baltimore, MD 21224, USA. Tel.: +1 410 558 8162; fax: +1 410 558 8157.
E-mail address: vbohr@nih.gov (V.A. Bohr).

0197-4580/$ – see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.neurobiolaging.2014.01.004

Table 1
Patient sample information obtained from Harvard Brain Bank

Sample	PMI	Background	Age (y)	Braak stage	Sex	Area	Source
AN14331	17.18	AD	82	5	F	Inferior parietal	Harvard Brain Bank
AN14184	15.42	AD	78	5	M	IPL	Harvard Brain Bank
AN06848	22.84	AD	60	6	F	IPL	Harvard Brain Bank
AN12633	21.08	AD	77	5	F	IPL	Harvard Brain Bank
AN08341	20.5	AD	74	5	M	IPL	Harvard Brain Bank
AN10763	13.5	AD	62	6	M	IPL	Harvard Brain Bank
AN11017	23.12	Control	67	NA	M	IPL	Harvard Brain Bank
AN01077	19.38	Control	58	NA	F	IPL	Harvard Brain Bank
AN12916	25.43	Control	77	NA	M	IPL	Harvard Brain Bank
AN00349	17.58	Control	79	NA	F	IPL	Harvard Brain Bank
AN18592	24.42	Control	82	NA	F	IPL	Harvard Brain Bank
AN10212	20.53	Control	74	NA	M	IPL	Harvard Brain Bank

Key: AD, Alzheimer's disease; F, female; IPL, inferior parietal; M, male; NA, not applicable; PMI, postmortem interval.

(Wilson and Bohr, 2007). Interestingly, we recently showed that loss of NEIL1, a versatile DNA glycosylase, in mice led to defective spatial memory retention during Morris water-maze tests (Canugovi et al., 2012). Furthermore, loss of NEIL1 delayed motor function recovery postischemic reperfusion injury. These mice showed decreased incision activity in both whole tissue and mitochondrial extracts for 5-hydroxyuracil (5OHU), one of the major substrates for NEIL1. These results suggest that loss of DNA repair may also contribute to brain dysfunction and pathology.

In this study, we examined the relative activity levels of BER components in the inferior parietal (IPL) region of postmortem AD and age-matched control brains. IPL is one of the most affected areas impacted by AD pathology, and the relative ratio of oxidatively damaged bases per number of normal bases is high in this region (Gabbita et al., 1998). Here, we studied the activities of several enzymes of the BER pathway including DNA glycosylases, abasic site cleavage (AP endonuclease), gap filling (polymerase), and nick-sealing ligase. We found a deficiency in 5OHU incision and ligase activity in AD mitochondrial samples but no change in the uracil incision, abasic site cleavage, or gap-filling polymerase steps.

2. Materials and methods

2.1. Brain samples and source

The samples used in this study were obtained from Harvard Brain Tissue Resource Center (Belmont, MA, USA). All samples belong to Braak stage 5 or 6. The samples were isolated and stored within ~24 hours after death (Table 1). The samples were derived from part of IPL region of the brains. The sample size (n) is 6 for both control and AD brains. Details of the patients are listed in Table 1.

2.2. Oligonucleotide substrates

All oligonucleotides were ordered from IDT (San Diego, CA, USA). The oligonucleotide sequence and location of the specific lesions can be found in Table 2.

2.3. Tissue fractionation and lysate preparation

Whole tissue lysates were prepared as described previously (Weissman et al., 2007). Mitochondrial fractionation was

Table 2
Names and sequences of oligonucleotides used in the study

Name	Sequence
5OHU-in bubble-51mer	5'-GCTTAGCTTGGAATCGTATC-ATGTA**5**ACTCG-TGTGCCGTGTAGACCGTGCC-3' 3'-CGAATCGAACCTTAGCATAG–GCACCCGACAA-ACACGGCACATCTGGCACGG-5'
Nick-1-91mer	5'-TAATTAATGCTTGTAGGACATAATAATAACAATTGAATGTCT**G-OH/P-C**ACA … 3'-ATTAATTACGAACATCCTGTATTATTATTGTTAACTTACAGA **(C)** GTGT … … GCCACTTTCCACACAGACATCATAACAAAAAATTTCCACCAAAC-3' … CGGTGAAAGGTGTGTCTGTAGTATTGTTTTTTAAAGGTGGTTTG-5'
Nick-2-91mer	5'-TAATTAATGCTTGTAGGACATAATAATAACAATTGAATGTCT**G-OH/OH-C**ACA … 3'-ATTAATTACGAACATCCTGTATTATTATTGTTAACTTACAGA **(C)** GTGT … … GCCACTTTCCACACAGACATCATAACAAAAAATTTCCACCAAAC-3' … CGGTGAAAGGTGTGTCTGTAGTATTGTTTTTTAAAGGTGGTTTG-5'
U-91mer	5'-TAATTAATGCTTGTAGGACATAATAATAACAATTGAATGTCT **(U)** CACA … 3'-ATTAATTACGAACATCCTGTATTATTATTGTTAACTTACAGA **(C)** GTGT … … GCCACTTTCCACACAGACATCATAACAAAAAATTTCCACCAAAC-3' … CGGTGAAAGGTGTGTCTGTAGTATTGTTTTTTAAAGGTGGTTTG-5'
THF-91mer	5'-TAATTAATGCTTGTAGGACATAATAATAACAATTGAATGTCT **(F)** CACA … 3'-ATTAATTACGAACATCCTGTATTATTATTGTTAACTTACAGA **(C)** GTGT … … GCCACTTTCCACACAGACATCATAACAAAAAATTTCCACCAAAC-3' … CGGTGAAAGGTGTGTCTGTAGTATTGTTTTTTAAAGGTGGTTTG-5'
GAP-91mer	5'-TAATTAATGCTTGTAGGACATAATAATAACAATTGAATGTCT**G**CACA … 3'-ATTAATTACGAACATCCTGTATTATTATTGTTAACTTACAGA **( )** GTGT … … GCCACTTTCCACACAGACATCATAACAAAAAATTTCCACCAAAC-3' … CGGTGAAAGGTGTGTCTGTAGTATTGTTTTTTAAAGGTGGTTTG-5'

5OHU, a 5-hydroxyuracil represented by 5′ in a bubble shown by dashes; nick-1, a ligase-specific nick with a 3′-OH and 5′-P groups on the DNA ends within the nick; nick-2, 3′ and 5′-OH groups on the DNA ends within the nick, this was made by polymerase incorporation of a nucleotide within a gap substrate in the last row. Bold letters highlight the specific lesions and their location in the oligomeric DNA substrates tested in this study.
Key: GAP, a single-nucleotide gap; THF, a tetrahydrofuran abasic site analog; U, a uracil.