DNA base excision repair activities in mouse models of Alzheimer's disease

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Abstract
Alzheimer’s disease (AD) has been correlated with elevated levels of oxidative DNA damage. Base excision repair (BER) is the main repair pathway for the removal of oxidative DNA base modifications. We have recently found significant functional deficiencies in BER in brains of sporadic AD and amnestic mild cognitive impairment patients. In this study we tested whether altered BER activities are associated with appearance of symptoms in different brain regions of pre-symptomatic and symptomatic mice harboring mutant APP alone or in combination with Tau and PS1. Our results suggest that unlike in humans, the development of AD-like pathology in the studied mouse models is not associated with deficiencies in BER.

Keywords
Alzheimers disease; DNA repair; oxidative stress; oxidative DNA damage

Alzheimer's disease (AD) has been associated with elevated oxidative DNA damage in the human brain (Gabbita et al., 1998). Base excision repair (BER), the main repair pathway for the removal of oxidative base modifications from DNA, plays a vital role in the development and maintenance of the central nervous system (Fishel et al., 2006). The first step in BER is the removal of the damaged base by substrate-specific DNA glycosylases to generate an abasic site which is then cleaved by an AP lyase or AP endonuclease (APE). This is followed by incorporation of one or several nucleotides into the gap by DNA polymerase and strand ligation by a DNA ligase. We have recently found significant functional deficiencies in BER in brains of sporadic AD and amnestic mild cognitive impairment (MCI) patients due to limited DNA base damage processing by DNA glycosylases and reduced DNA synthesis capacity by polymerase β (polβ) (Weissman et al., 2007). However, the question of how BER deficiency is involved in the progression of AD has yet to be answered.

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The study of DNA repair capacity in the human AD brain is limited to scarcely available autopsy tissue samples. However, mouse models may allow assessing these activities starting at very early stages of the disease and throughout its progression. The aim of this study was to ascertain whether altered BER activities occur in an age-dependent manner in AD mouse models and to gain insight into such possible alterations in the early stages of the disease. We therefore compared BER activities in different brain regions from pre-symptomatic (3 months old) and symptomatic (10 months old) APP mice overexpressing a mutant beta amyloid precursor protein (APP<sub>Swe</sub>), and in 3xTgAD mice harboring the APP<sub>Swe</sub>, presenilin 1 (PS1<sub>M146V</sub>), and Tau<sub>P301L</sub> transgenes. Male C57BL/6 mice served as controls for polβ and APE1 activity assays. To eliminate possible “premature aging” effects in AD mice, we used 6 month- and 20 month old wild type mice for comparison of polβ and APE1 activities. Two independent lysates, each made using brain tissue from three mice, were prepared for every brain region. Lysates were assayed twice for each activity, and two reactions were conducted for each assay. The activities of DNA glycosylases and APE1 were measured by employing a set of DNA cleavage assays with 5'-32P-labeled-oligonucleotides containing either 8-oxoG, 5-OH-C, uracil, or tetrahydrofuran (THF), an abasic site analog (de Souza-Pinto et al., 2001; de Souza-Pinto et al., 2004) (Suppl. Table 1). For polβ gap-filling reactions, we used an unlabeled double-stranded substrate containing a single-nucleotide gap (Weissman et al., 2007).

Our results show that 8-oxoG incision activity did not change significantly in the frontal cortex (FC), hippocampus (HIP), cerebellum (CE) or brain stem (BS) of older, symptomatic APP mice relative to young, pre-symptomatic mice. However, a small, yet significant decrease was detected in the caudate nucleus (CN) of APP mice. (Suppl. Fig. 1B). Similarly, this activity in 3xTgAD mice did not change in the FC, HIP or BS of symptomatic mice relative to pre-symptomatic mice, whereas lower activities were detected in the CN and CE (Suppl. Fig. 1C). 5-OH-C incision activity did not differ significantly between pre-symptomatic and symptomatic mice in any of the brain regions of either AD model (Suppl. Figs. 1E, 1F). Uracil incision activity did not change significantly in CN, FC, HIP or CE of symptomatic APP mice compared with pre-symptomatic mice, although in the BS, this activity was significantly decreased (Suppl. Fig. 2B). In 3xTgAD mice, uracil incision activity did not change significantly in the tested brain regions of symptomatic relative to pre-symptomatic mice, except in the CN (Suppl. Fig. 2C). AP-site incision by APE1 (Suppl. Fig 2E, 2F) as well as single-nucleotide gap-filling activity, a primary function of polβ (Suppl. Fig 3B, 3C), did not change significantly with age in any brain region of either WT or 3xTgAD mice. Moreover, similar values for AP-site incision and Polβ activities were found in the WT mice, compared to the 3xTgAD mice (Suppl. Fig 2E, 2F, 3B, 3C).

8-oxoG, an abundant mutagenic oxidized purine, and 5-OH-C, an oxidized pyrimidine, were previously shown to accumulate to a greater extent in affected human AD brain regions relative to aged matched controls (Gabbita et al., 1998; Wang et al., 2005). Oxoguanine DNA glycosylase (OGG1), the primary enzyme for the repair of 8-oxoG (Klungland et al., 1999; de Souza-Pinto et al., 2001), was found to be decreased in expression and activity in human AD brain tissues (Lovell et al., 2000; Iida et al., 2002; Weissman et al., 2007). Our present results show that in AD mice, 8-oxoG incision activity was not significantly altered in disease-associated brain regions (e.g. HIP or FC), and incision activity of 5-OH-C remained unaffected. Similarly, uracil incision activity, which was found reduced in brain of human AD and MCI patients (Weissman et al., 2007), was only slightly altered in AD mice brain regions not typically associated with the disease.

The association of altered APE1 with human AD pathology has been suggested in protein expression studies (Davydov et al., 2003; Tan et al., 1998). However, APE1 activity in brains

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of AD patients was found similar to non-AD controls (Weissman et al., 2007). Accordingly, we report here that AP-site incision activity did not alter significantly in brain regions of symptomatic relative to pre-symptomatic 3xTgAD animals, nor did it change with age in WT mice. Moreover, the activity levels in 3xTgAD brain regions were similar to those of WT mice, suggesting that this step in BER is not affected by the disease in the tested mouse model.

DNA pol β protects cells against the cytotoxicity of oxidative DNA damage (Chen et al., 1998) and plays a role in genome maintenance in aging and carcinogenesis (Cabelof et al., 2006). Importantly, mice lacking pol β display neonatal lethality with abnormal neurogenesis (Sugo et al., 2000), suggesting a critical role in neuronal survival. We show here that single nucleotide gap-filling activity, a function of polβ, did not differ significantly between pre-symptomatic and symptomatic 3xTgAD mice, and did not change with age in WT mice. This finding differs from the significant deficiency in gap-filling activity observed in human AD (Weissman et al., 2007). Interestingly, one major difference between the mouse models of AD and humans is that, in spite of accumulation of plaques and tangles, mice do not display neuronal degeneration. Since we did not observe decreased pol β activity in 3xTgAD mice, it is possible that the normal level of pol β in the brain of this transgenic mouse model protects it from Aβ-induced neuron death. In summary, this study explored possible alterations in BER activities in AD mouse models. Our results suggest that the development of AD-like pathology in the studied mouse models is not associated with deficiencies in BER. Expanded methodology and references are available in the supplementary data.

Supplementary Material
Refer to Web version on PubMed Central for supplementary material.

References
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