Correlated Oxidative Stress and Mitochondrial Abnormalities in Aging are Discontinuous with Alzheimer's Disease

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ABSTRACT

Oxidative stress and mitochondrial damage precede Alzheimer's disease (AD) hallmark pathologies, neurofibrillary tangles (NFT), and senile plaques. Mitochondria's exact role in oxidation of pyruvate and NADH play a critical role in oxidative damage. We conducted this study to identify the relationship of oxidized RNA, 8-OHG biomarker, and mtDNA accumulation in AD and aging individuals. Abnormalities were examined by using densitometry of hippocampal pyramidal neurons: mtDNA accumulation as a marker of mitophagy and oxidative damage by 8-OHG. Among aging individuals, 8-OHG and mtDNA accumulation were highly correlated (R^2 = 0.87, p=0.0007). While both 8-OHG and mtDNA were at higher levels in AD individuals, they were uncorrelated (R^2 = 0.4418, p=0.07). In AD individuals, 8-OHG was inversely correlated with amyloid-β, while in aging, there was no significant correlation. These results suggest the discontinuity of similarities between aging and AD. These findings also indicate that the onset of AD is marked by a pleotrophic change in oxidative stress, one characterized by a change from mitochondria degeneration to amyloid-β independency.

INTRODUCTION

- Alzheimer's disease (AD) occurs 1-in-3 seniors over 65 y/o
- Costs USA $277 billion.
- No way to identify and diagnose AD before autopsy with 100% confidence.
- Many studies have pointed to the correlation of AD and aging.
- AD hallmarks are senile plaques, consisting of amyloid-β protein, and neurofibrillary tangles.
- Aging is characterized by oxidative stress and mitochondrial changes.

OBJECTIVE

To understand the relationships of AD and aging by focusing on oxidized RNA (8-OHG), mitochondrial DNA (mtDNA), and their relation to amyloid-β.

HYPOTHESIS

We hypothesize that the inverse relationship of oxidative stress (8-OHG) to amyloid-β is due to a divisional and discontinuation of AD with diseases associated with aging because of a deviation in the accumulation of abnormal mitochondria.

METHODOLOGY

- Brain tissue collected from 23 clinically confirmed AD individuals
  - 9 males/14 females; ages 57-93 y/o
- Brain tissue collected from 27 controls without AD
  - 19 males/8 females; 3-86 y/o; avg. 60 y/o
- Hippocampal pyramidal neurons fixed in methacarn (6:3:1; methanol, chloroform, acetic acid) at 4˚ C for 16 hours
- Dehydrate with degraded ethanol, xylene, and inserted in paraffin
- 6µm sections cut and mounted on silane coated glass slides
- Hydration with graded ethanol and incubate 30 minutes with 3% H_2O_2 in methanol
- Incubate 30 minutes in 10% normal goat serum in Tris-buffered saline (150mM Tris-HCl, 150 mM NaCl, pH 7.6)
- Intensity of immunoreactions were measured with Quantimet 570C Image Processing and Analysis System linked to COHU Solid State Camera put on Leitz Laborlux 12 ME ST microscope
- Densitometry measured by average optical density of cytoplasm and nucleus

RESULTS

Aging

- As 8-OHG increases, Aβ increases; R^2=0.4684, p=0.09
- As 8-OHG increases, mtDNA accumulation increases; R^2=0.8717, p=0.0007
- mtDNA levels were lower; p<0.0001
- 8-OHG levels were lower; p<0.00001

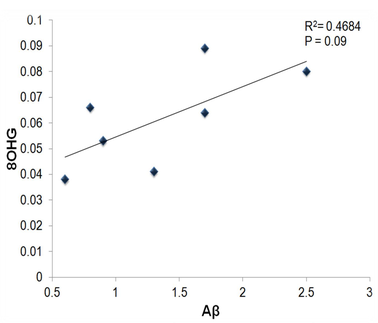

Figure 1: In aging, there is a positive relation between oxidative and Aβ, but not statistically significant.

AD

- As 8-OHG increases, Aβ decreases nonlinearly; $y=1.0651x^{-1.41}$, R^2=0.5956, p=0.0007
- 8-OHG and mtDNA are uncorrelated

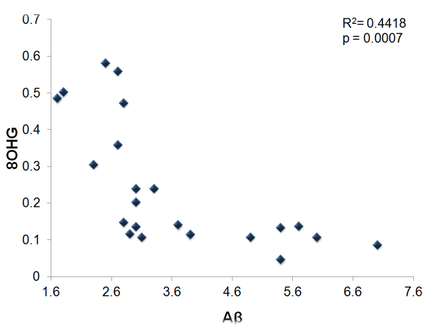

Figure 2: In AD, oxidative stress is inversely related to Aβ, which is significant.

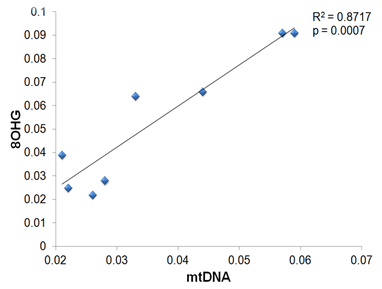

Figure 3: In aging, there is a direct relation between oxidative stress and mtDNA, which is highly correlated and statistically significant.

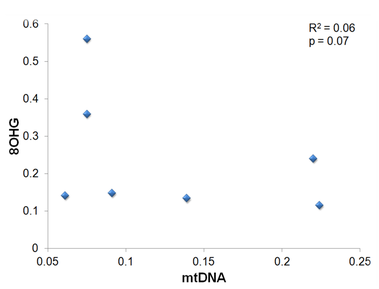

Figure 4: In AD, oxidative stress and mtDNA are highly uncorrelated.

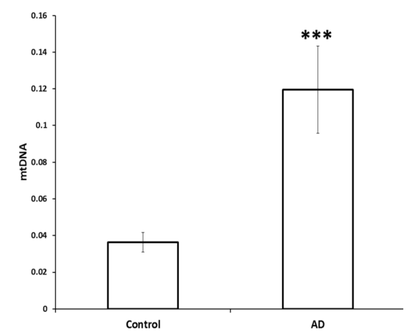

Figure 5: In AD, mtDNA levels were statistically higher than in controls; ***p<0.0001

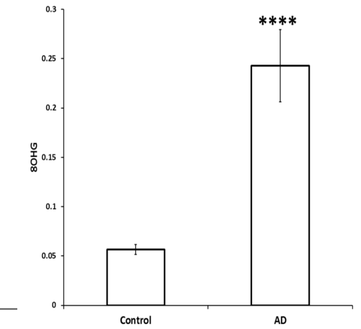

Figure 6: In AD, 8-OHG levels were significantly higher than in controls; ****p<0.00001

DISCUSSION & CONCLUSION

- ❖ Oxidative stress and mitochondrial dysfunction characterizes aging and to a degree AD, but this characterization moves away from each other as AD progresses.
- ❖ AD lacks the ability to regulate oxidative stress and accumulation of mtDNA; hence amyloid-β deposits are present, as a mechanism of protection from oxidative damage.
- ❖ AD progresses to compensate for lack of oxidative stress and mtDNA seen in normal aging.
- ❖ Changes in AD brain resembles changes following TBI, suggesting AD may be the outcome of the brain's inability to recover and rehabilitate from oxidative stress, thus becoming a disease not related to aging, but rather a response to injury.

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