DNA damage increases secreted $A\beta_{40}$ and $A\beta_{42}$ in neuronal progenitor cells: relevance to Alzheimer's Disease

Starr Welty[1], Amantha Thathiah[1,2,3], Arthur Samuel Levine[1,2]

[1]Department of Neurobiology, University of Pittsburgh School of Medicine, Pittsburgh, PA, [2]University of Pittsburgh Brain Institute, Pittsburgh, PA; [3]Pittsburgh Institute of Neurodegenerative Diseases, University of Pittsburgh, Pittsburgh, PA

ABSTRACT

Background:
Recent studies suggest a strong association between neuronal DNA damage, elevated levels of amyloid-β (Aβ), and regions of the brain that degenerate in Alzheimer's Disease (AD).

Objective:
To investigate the nature of this association, we tested the hypothesis that extensive DNA damage leads to an increase in $A\beta_{40}$ and $A\beta_{42}$ generation.

Methods:
We utilized an immortalized human neuronal progenitor cell line (NPCs), ReN VM GA2. NPCs or 20 day differentiated neurons were treated with hydrogen peroxide or etoposide and allowed to recover for designated times. Sandwich ELISA was used to assess secreted $A\beta_{40}$ and $A\beta_{42}$. Western blotting, immunostaining, and neutral comet assay were used to evaluate the DNA damage response and processes indicative of AD pathology.

Results:
We determined that global hydrogen peroxide damage results in increased cellular $A\beta_{40}$ and $A\beta_{42}$ secretion 24 hours after treatment in ReN GA2 NPCs. Similarly, DNA double strand break (DSB)-specific etoposide damage leads to increased $A\beta_{40}$ and $A\beta_{42}$ secretion 2 hours and 4 hours after treatment in ReN GA2 NPCs. In contrast, etoposide damage does not increase $A\beta_{40}$ and $A\beta_{42}$ secretion in post-mitotic ReN GA2 neurons.

Conclusion:
These findings provide evidence that in our model, DNA damage is associated with an increase in Aβ secretion in neuronal progenitors, which may contribute to the early stages of neuronal pathology in AD.

INTRODUCTION

Alzheimer's Disease (AD) is the most common form of dementia, representing roughly 50 million annual worldwide cases. Although numerous treatments have been proposed, including monoclonal antibodies specific for Aβ and tau, all have failed to show significant efficacy in clinical trials, with no evidence to date that synaptic and neuronal loss in the hippocampus and cerebral cortex in later stages of AD is preventable. Thus, there is a critical need to improve our understanding of the early mechanisms at play in AD. One such mechanism may relate to DNA damage, a common consequence of oxidative stress.

Oxidative stress is defined as an imbalance between the production and clearance of reactive oxygen species (ROS). Oxidative damage can lead to DNA double strand breaks (DSBs), the most deleterious of DNA lesions. Efficient DSB repair is especially important in neuronal progenitors which differentiate into post-mitotic neurons. There is a significant dearth of knowledge on how AD initially develops, especially regarding neural stem cells and neuronal precursors/progenitors. Although many studies suggest a causal association between neuronal oxidative DNA damage and the accumulation of Aβ, a direct link has not been established experimentally.

To test the hypothesis that DNA damage directly induces Aβ secretion, here we determined the effect of global oxidative damage (hydrogen peroxide) and specific DSB DNA damage (etoposide) on Aβ generation in the ReN GA2 cell line, an immortalized human neuronal progenitor cell (NPC) line that can be differentiated into neurons. We sought to determine whether mitotic cells and post-mitotic neurons are differentially affected by DNA damage, with a consequent effect on Aβ generation.

RESULTS

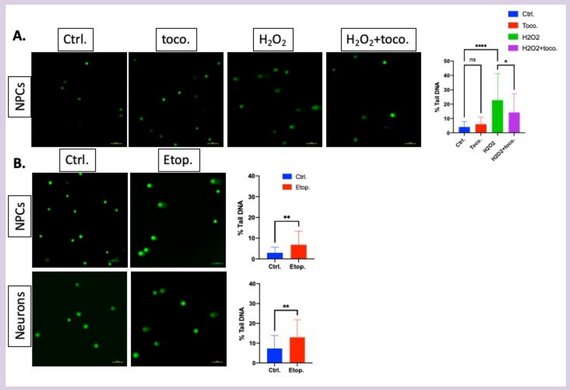

Fig 1. Hydrogen peroxide and etoposide damage induce DNA DSBs differentially in ReN GA2 NPCs and neurons. A. Cultures of ReN GA2 NPCs were assessed for DSB levels by neutral comet assay with and without 5 μM α-tocopherol pre-treatment for 0.5 hr, treated with and without 2.5 μM peroxide for 0.5 hr. % tail DNA was quantified using 30 nuclei per experiment (100x tail DNA intensity/cell DNA intensity). **B.** Cultures of ReN GA2 NPCs and 20 day differentiated neurons were assessed for DSB levels by neutral comet assay with and without 6 hr 10 μM etoposide treatment. % tail DNA was quantified using 30 nuclei per experiment (100x tail DNA intensity/cell DNA intensity). Error bars represent means ± SD of three separate experiments, and the P values were determined using one-way ANOVA and Tukey's multiple comparisons test, or Students T test. *P<0.05, **P<0.01, ****P<0.0001.

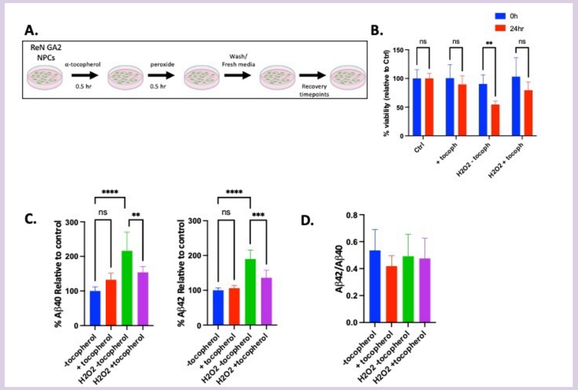

Fig 2. Hydrogen peroxide damage increases Aβ40 and Aβ42 secretion in ReN GA2 NPCs, an effect that is mitigated by α-tocopherol treatment. A. Schematic of ReN GA2 NPC experimental design. ReN GA2 NPCs were treated with and without 5 μM α-tocopherol prior to treatment with and without 2.5 μM hydrogen peroxide for 0.5 hr and allowed to recover for 24 hr. (Schematic created in BioRender, 2021.) **B.** Cell viability was determined after indicated treatment and 24 hr recovery time. **C.** Percent secreted Aβ40 & Aβ42 relative to controls after designated treatments and 24 hr recovery time. **D.** Aβ42/Aβ40 ratio calculated from raw values. Error bars represent means ± SD of three separate experiments, and the P values were determined using 2-way ANOVA and Tukey's multiple comparisons test. **P<0.01, ***P<0.001, ****P<0.0001.

RESULTS

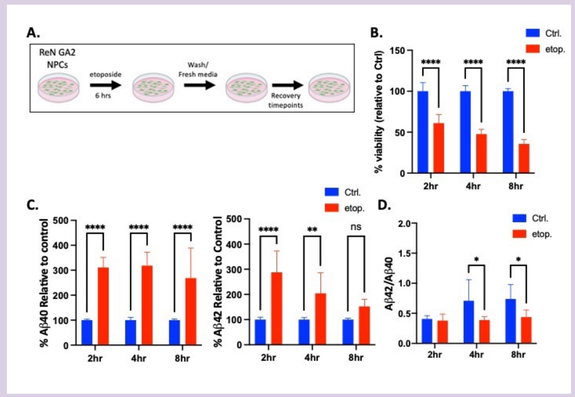

Fig 3. Etoposide damage increases Aβ40 and Aβ42 secretion after 2 hr and 4 hr recovery in ReN GA2 NPCs. A. Treatment schematic for ReN GA2 NPCs treated with and without 10 μM etoposide for 6 hr and allowed to recover for designated times. (Schematic created in BioRender, 2021.) **B.** Percent cell viability after 6 hr etoposide treatment and designated recovery times. **C.** Percent secreted Aβ40 & Aβ42 relative to controls after designated treatments and recovery times. **D.** Aβ42/Aβ40 ratio calculated from raw values. Error bars represent means ± SD of three separate experiments, and the P values were determined using 2-way ANOVA and Tukey's multiple comparisons test. *P<0.05, **P<0.01, ****P<0.0001, ns: not significant.

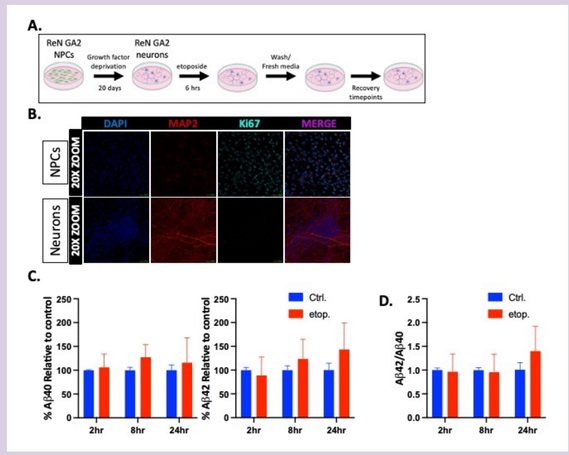

Fig 4. Etoposide damage does not increase Aβ40 and Aβ42 secretion in differentiated ReN GA2 neurons. A. Treatment schematic for differentiating ReN GA2 neurons and treatment with and without 10 μM etoposide for 6 h and designated recovery times. (Schematic created in BioRender, 2021.) **B.** Immunofluorescent staining of ReN GA2 NPCs and 20-day differentiated neurons with antibodies for anti-MAP2 (neuron) and anti-Ki67 (proliferation marker). **C.** Percent secreted Aβ40 & Aβ42 relative to controls after 6 hr etoposide treatment and designated recovery times. **D.** Aβ42/Aβ40 ratio calculated from raw values. Error bars represent means ± SD of three separate experiments.

RESULTS

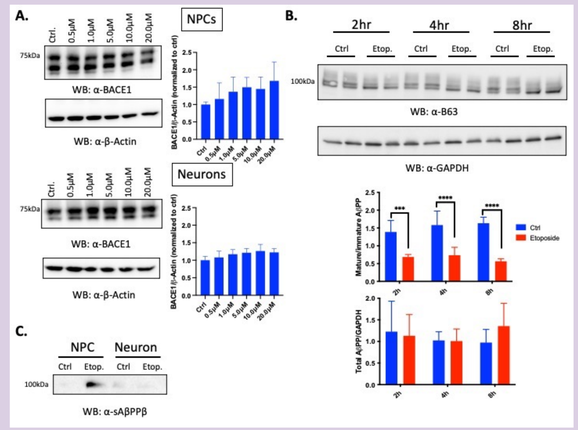

Fig 5. DSBs increase sAβPPβ, but not total AβPP in ReN GA2 NPCs. A. Western blot of endogenous BACE1 expression in ReN GA2 NPCs and 20 day differentiated neurons treated with and without designated concentrations of etoposide for 6 hr and allowed to recover for 2 hr. **B.** Western blot of endogenous AβPP expression in ReN GA2 NPCs treated with and without 10 μM etoposide for 6 hr and allowed to recover for designated times. **C.** Western blot of sAβPPβ in ReN GA2 NPCs and 20 day differentiated neurons with and without 10 μM of etoposide treatment for 6 hr and allowed to recover for 2 hr. Error bars represent means ± SD of two to three separate experiments, and the P values were determined using two-way ANOVA and Tukey's multiple comparisons test. ***P<0.001, ****P<0.0001.

CONCLUSIONS

- Hydrogen peroxide damage increases Aβ40 and Aβ42 secretion in ReN GA2 NPCs, mitigated by α-tocopherol.
- Etoposide damage increases Aβ40 and Aβ42 secretion in ReN GA2 NPCs, but not post-mitotic neurons.
- Etoposide induced DSBs increase sAβPPβ, but not total AβPP or BACE1 in ReN GA2 NPCs.

ACKNOWLEDGEMENTS

This study was supported by R01AG058851, a NINDS T32 NS086749 Training Grant, and institutional cash funds.

REFERENCES

- Welty S, Thathiah A, Levine AS. DNA Damage Increases Secreted Aβ40 and Aβ42 in Neuronal Progenitor Cells: Relevance to Alzheimer's Disease. J Alzheimers Dis. 2022 May 9. doi: 10.3233/JAD-220030. PMID: 35570488.