AAP DISTRICT VIII SECTION ON NEONATAL PERINATAL MEDICINE

2021 ANNUAL CONFERENCE **ORIGINAL RESEARCH** (BASIC SCIENCE or CLINICAL)
ABSTRACT SUBMISSION FORM

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Signature: _____Sarah Kolnik_____ Date: ____2/4/2021_____

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DEADLINE FOR RECEIPT OF ABSTRACT IS FEBRUARY 19, 2021. Submissions will be accepted for either poster or oral presentation. Authors will be notified of acceptance and format for presentation (poster or poster symposium) by **March 12, 2021.**

Title: Vitamin E (Vit E) Mitigates Inflammatory and Oxidative Stress Responses in a Ferret Organotypic Brain Slice Model of Hypoxia Ischemia (HI)

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Background: The ferret brain is an excellent mid-sized animal in which to study HI, a significant contributor to neurological injury in neonates. Vit E, an essential fat-soluble antioxidant acts by scavenging free radicals and reducing oxidative stress and inflammation in rodent models and in neonates.

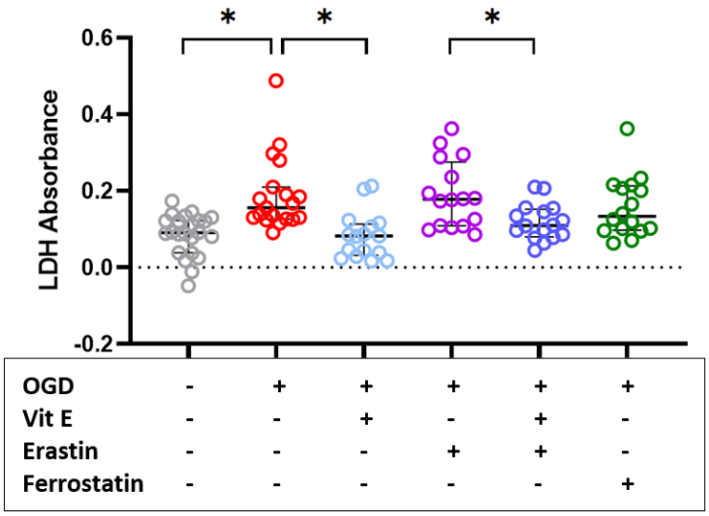
Objective: To assess the effects of Vit E after oxygen-glucose deprivation (OGD), in *ex vivo* model of HI, in ferret organotypic brain slices, which retain much of the cellular and structural architecture of the intact brain. We hypothesized 1) Vit E would decrease cytotoxicity, inflammation and oxidative stress in OGD-exposed brain slices 2) Erastin, a promotor of oxidative stress, would further exacerbate the deleterious effects of OGD which would then be reversed with the treatment of Vit E via a mechanism similar to ferrostatin, an inhibitor of oxidative stress.

Design/Methods: Term-equivalent ferrets were sacrificed at postnatal (P) day 21-23, and 300µM whole hemisphere slices were obtained using a vibratome. Slices were exposed during a 24h rest period to either non-treated (NT) conditions, 25IU/kg of Vit E, or Erastin a promotor of oxidative stress. After 24h rest, slices were exposed to either control conditions (NT) or 2h of OGD with or without Vit E (25 IU/kg), Erastin (10µM), or Ferrostatin (1µM). Relative cytotoxicity was determined using an LDH assay, cell death was quantified via confocal microscopy of propidium iodide (PI) stained cells, cellular glutathione (GSH) levels were measured, and target genes responsive to oxidative stress (CHAC, PTGES2, SLC7A11, GCLM, HMOX1) and inflammation (IL-1beta, IL-6, IL-8, IL-10, TNF-alpha) were evaluated by qRT-PCR.

Results: OGD increased LDH levels compared to control, significantly increased cell death from 35% to 60% and decreased GSH **(Figure 1,2, 3)**. OGD significantly increased markers of oxidative stress CHAC1 and SLC7A11, as well as markers of inflammation TNF alpha and IL-8 **(Figure 4,5)**. Vit E reversed the increase in LDH caused by OGD, decreased cell death to similar to control levels, and increased GSH to control levels (**Figure 1,2,3**). Vit E also decreased markers of inflammation and oxidative stress **(Figure 4,5)**. Erastin+OGD increased LDH levels which were significantly reduced by Vit E **(Figure 1).** The addition of Vit E to OGD+Erastin decreased cell death from 70% to 54% (**Figure 2**). OGD+Ferrostatin+Vit E had similar levels of cytotoxicity after OGD. (**Figure 1,2**).

Conclusion: Results from this pilot study in the ferret whole hemisphere OGD slice culture support the premise that Vit E may be neuroprotective by acutely decreasing inflammation and oxidative stress after HI brain injury.

Figure 1: Cytotoxicity of brain slices determined 24h post OGD using a LDH assay (n = minimum 16 slices per group). OGD increased LDH levels compare to control (p=0.002) whereas addition of Vit E to OGD decreased LDH levels significantly back down to control level (p=0.030). Erastin increased cytotoxicity which was significantly reduced by addition of Vit E (p = 0.012). Vit E and Ferrostatin had similar levels of LDH (p=0.26). *denotes significance compared to NT (p<0.05). Mann-Whitney U-test.



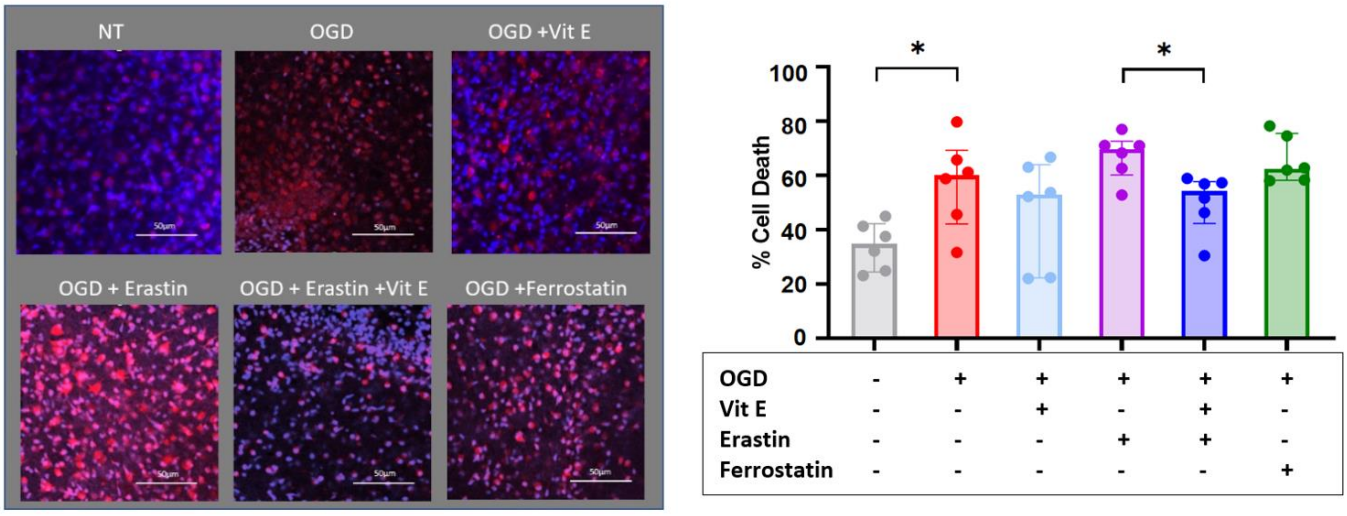


Figure 2: A. Propidium iodide-stained nuclei indicating dying cells (red) and healthy cell nuclei DAPI (blue). Representative 20x magnification images are shown in the corpus callosum region that reflect median of each condition. **B.** Percentage of cells co-localized with PI in 20x images of the corpus callosum region. OGD significantly increased cell death from 35% to 60% (p=0.024) whereas addition of Vit E to OGD decreased cell death more similar to controls (p=0.39). The addition of Vit E to Erastin decreased cell death from 70% to 54% (p = 0.015) *n* per group 6. *denotes significance compared to NT (p<0.05). Mann-Whitney U-test.

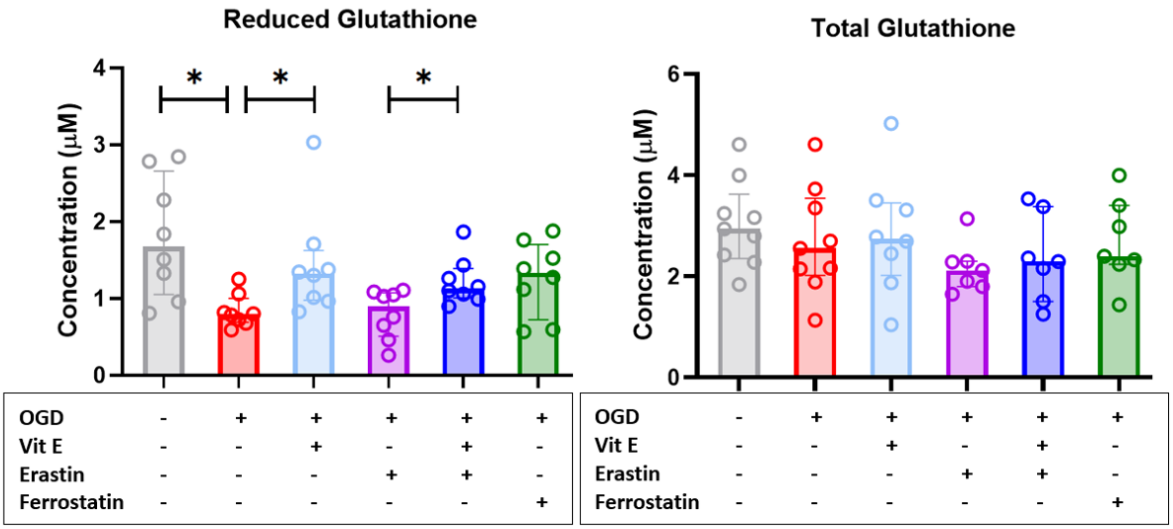


Figure 3: Levels of reduced glutathione and total glutathione were determined 24h post OGD (n=8 slices each condition). Reduced glutathione was significantly decreased in OGD (p =0.0030)whereas those treated with Vit E recovered significantly (p=0.0047) Slices treated with Erastin had a lower level of reduced glutathione that was recovered with Vit E significantly (p =0.028). As expected, total glutathione in all groups did not vary significantly. *denotes significance compared to NT (p<0.05). Mann-Whitney U-test.

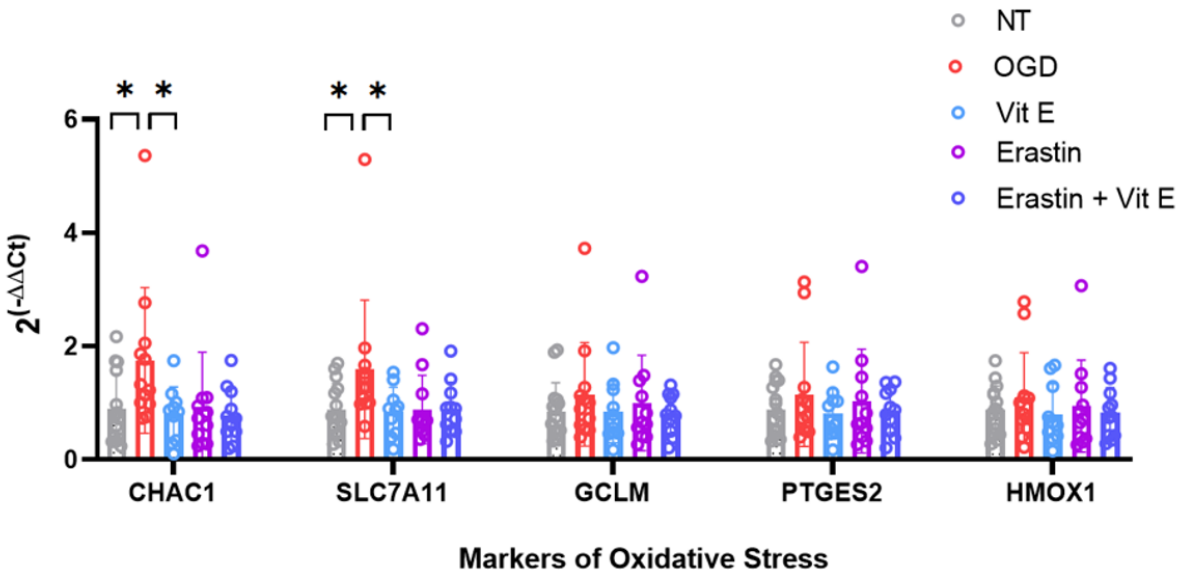


Figure 4 Trends of increased amplification of markers of oxidative stress after OGD which partially or fully normalized after Vit E treatment (n = minimum 10 slices per group). Significant increase in CHAC1 after OGD (p= 0.019) and decrease after Vit E treatment (p=0.017); similarly increase in SLC7A11 after OGD (p = 0.018) and decrease after Vit E treatment (p=0.016). Erastin compared to Erastin with Vit E did not have significant differences. *denotes significance compared to NT (p<0.05). Mann-Whitney U-test.

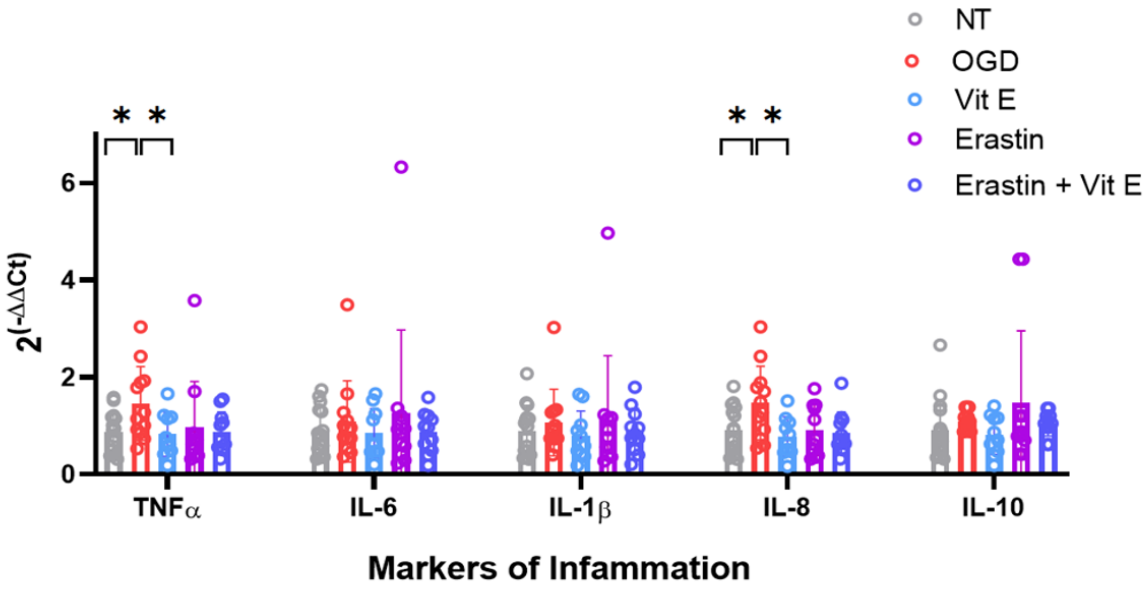


Figure 5 Trends of increased amplification of markers of inflammation after OGD which partially or fully normalized after Vit E treatment (n = minimum 10 slices per group). Significant increase in TNF alpha after OGD (p= 0.032) and decrease after Vit E treatment (p=0.043); similarly increase in IL-8 after OGD (p = 0.041) and decrease after Vit E treatment (p=0.017). Erastin compared to Erastin with Vit E did not have significant differences. *denotes significance compared to NT ($p<0.05$). Mann-Whitney U-test.