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Zika Virus Induces Apoptosis in Retinal Pigmented Epithelial Cells

Madison Kraus
University of Nebraska-Lincoln, madison.kraus@huskers.unl.edu

Philma Glora Muthuraj
University of Nebraska-Lincoln, philma@huskers.unl.edu

Sathish Kumar Natarajan
University of Nebraska-Lincoln, snatarajan2@unl.edu

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Department of Nutrition and Health Sciences, University of Nebraska- Lincoln

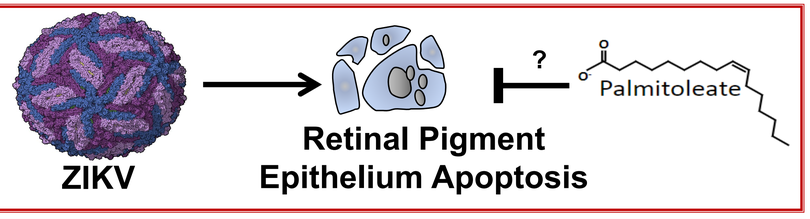
BACKGROUND

- Zika Virus (ZIKV), a mosquito-borne flavivirus is a single stranded positive sense RNA virus
- Although it was first identified in 1947, the recent 2015 epidemic in Brazil attracted international attention as it spread around the world
- In healthy individuals, ZIKV infection may present with mild flu-like symptoms
- ZIKV infection during pregnancy is extremely dangerous as the virus has the potential to spread *in vitro* or during delivery to the fetus causing birth defects including Microcephaly and Congenital Zika Syndrome
- A defect can be observed in the loss of retinal epithelial cells of a child's eyes that results in blind spots in their vision
- ZIKV-induced apoptosis in retinal epithelial cells has been observed

PURPOSE

The prevalence of Zika virus infection, including Brazil, has affected many newborns and the lives of families causing a great financial burden. The purpose of this UCARE project is to investigate a nutrient supplementation to prevent pregnant mothers and infants from the ZIKV infection.

HYPOTHESIS



METHODS

- The MR766 (African ZIKV strain) and PRVABC-59 (Asian strain) viral titers were determined by plaque assay using Vero cells
- Choriocarcinoma-derived trophoblast cells, JEG-3, JAR cells and human immortalized retinal pigment epithelial cells (ARPE-19) were infected with 0.1 multiplicity of infection (MOI) with MR766 or PRVABC-59 strain for 48-96h
- Cells were pretreated with100 µM or 200 µM of palmitoleate (PO), an omega-7 monounsaturated fatty acid or palmitate dissolved in isopropanol in 1% BSA containing media for 12 hours before infection and then infected with 0.1 MOI MR766 ZIKV
- Post-treatment: After ZIKV infection, cells were treated with 100 µM and 200 µM of palmitate (PA) or palmitoleate (PO) in 1% BSA containing media for 72-96 hours.
- Apoptosis was assessed by biochemical characteristic nuclear morphology changes with DAPI using epifluorescence microscope
- Caspase 3/7 activity were also measured and expressed as fold change compared to sham infection.

RESULTS

Palmitoleate protects ZIKV-induced Trophoblast Apoptosis

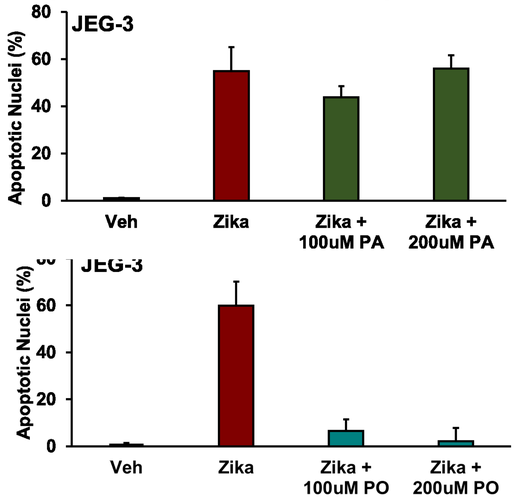


Figure 1: Palmitoleate vs. Palmitate : Palmitate, saturated fatty acid (C16) does not show significant protection from ZIKV PRVABC-59 strain infection. Post treatment of palmitoleate shows dramatic protection from ZIKV infection-induced apoptosis in JEG-3 cells. These data represents mean± standard error of mean (SEM) from 5 independent experiments. * P<0.05 compared to Veh, #P<0.05 compared Zika infection alone.

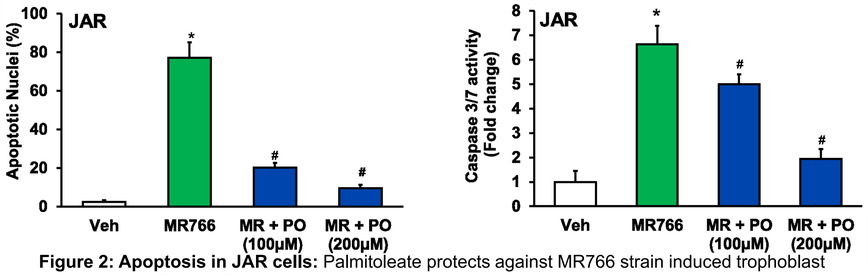


Figure 2: Apoptosis in JAR cells: Palmitoleate protects against MR766 strain induced trophoblast apoptosis in JAR cells. There is a significant decrease in apoptotic nuclei percent in cells protected with palmitoleate. These data represents mean ± standard error of mean (SEM) from 5 independent experiments. * P<0.05 compared to Veh, #P<0.05 compared MR766 infection alone.

Palmitoleate protects ZIKV-induced Retinal Pigment Epithelial cell Apoptosis

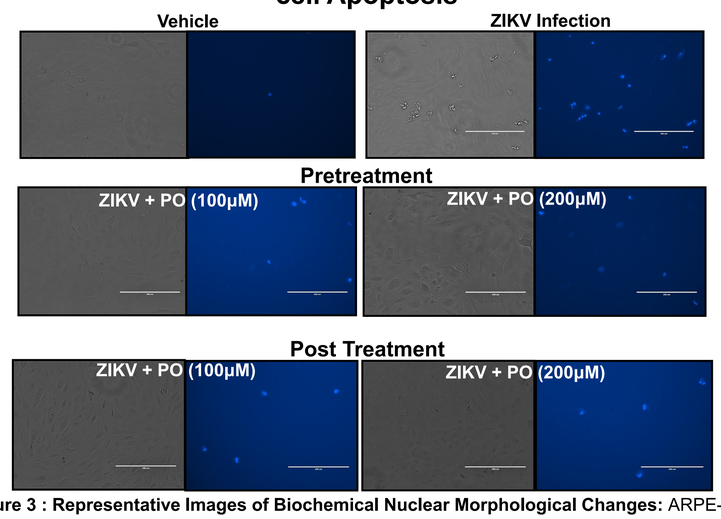


Figure 3 : Representative Images of Biochemical Nuclear Morphological Changes: ARPE-19 cells were treated with different concentrations of PO (100, and 200µM) prior to viral infection for 12 hours or following viral infection for 72 hours. With DAPI staining, a decrease in nuclear condensation and fragmentation is observed in cells treated with PO (A).

RESULTS

ZIKV-induced Retinal Pigment Epithelial cell Apoptosis were protected in Pre- and Post-treatment of Palmitoleate

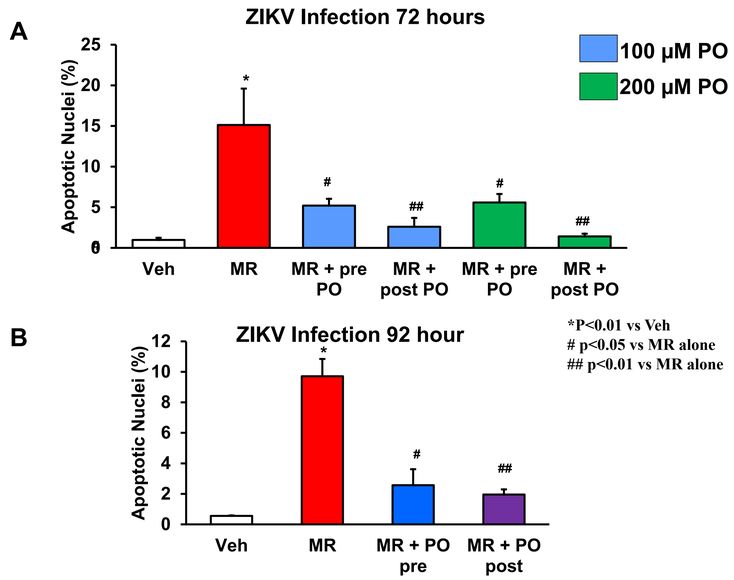
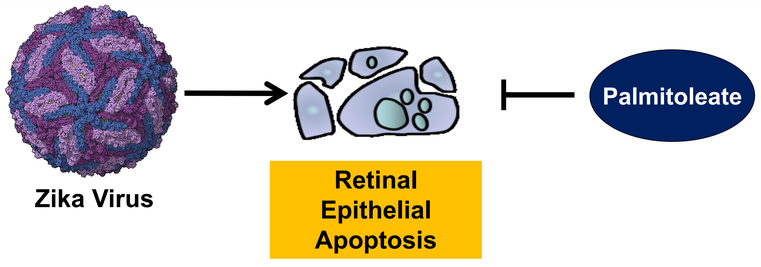


Figure 4 : Apoptosis: ARPE-19 cells infected with 0.1 MOI of MR766 showed significant increases in apoptotic nuclei percent. Cells pretreated for 12 hour with 100 µM or 200 µM PO and infected with 0.1 MOI MR766 showed a significant decrease in apoptotic nuclei percent. Cells post-treated with 100 µM or 200 µM PO show significant decrease in apoptotic nuclei percent (A). ARPE-19 cells infected with 0.1 MOI of strain MR766 for 96 hours and pretreated with 100 µM and post-treatment of PO show significant protection (B).

CONCLUSION



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