The Addition of Arachidin 1 or Arachidin 3 to Human Rotavirus-infected Cells Inhibits Viral Replication and Alters the Apoptotic Cell Death Pathway

Macie N. Mattila, Caleb M. Witcher, Rebekah Napier-Jameson, Hannah N. Lockwood, Josephine Taylor, Beatrice A. Clack, Judith M. Ball, Fabrisio Medina Bolivar, Rebecca D. Parr

1) Department of Biology, Stephen F. Austin State University, Nacogdoches, TX 75962. 2) Department of Pathobiology, Texas A&M University Commerce, Commerce, TX 75428. 3) Department of Biological Sciences and Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR 72401. *email: parrr1@sfasu.edu

Introduction

Rotavirus (RV) infections are a leading cause of severe gastroenteritis in infants and children under the age of five. There are two vaccines available in the United States and one in India that can be administered early in childhood, however they only protect against specific strains1. From our previous work, both arachidin-1 (A1) and arachidin-3 (A3) from peanut (Arachis hypogaea) hairy root cultures significantly inhibit simian RV replication2,3,4. The purpose of this study was to determine if a human intestinal cell line, HT29.f8, infected with a human RV, Wa, was affected by A1 and A3. Cell viability assays were utilized to determine if A1 and A3 affect the HT29.f8 cells with/without RV infections. At eighteen hours post infection (hpi), supernatants from the RV-infected HT29.f8 cells with/without the arachidins were used in plaque forming assays to quantify and compare the amount of infectious RV particles that are produced during an infection. Transmission electron microscopy (TEM) was used to visualize cell ultrastructure and individual RV particles. Additionally, quantitative real polymerase chain reactions (qRT-PCR) were performed to determine if A1 and A3 regulated cell death pathways in the HT29.f8 cell line. This data will guide our future studies to determine the antiviral mechanism(s) of action of A1 and A3.

Materials and Methods

1. Uninfected + no treatment
2. RV (Wa) + Worthington Trypsin (1µg/ml)
3. RV (Wa) + Worthington Trypsin (1µg/ml) + 20µM A1/3
4. Uninfected + 20µM A1/3

Results

Figure 1: Transmission Electron Microscopy images of RV-infected cells treated with A1 or A3 showing an increase in a similar size of the untreated or arachidin alone cells. The TEM images depicted changes of the ultrastructures of RV-infected HT29.f8 cells, and indicates apoptosis while RV-infected cells with the addition of A1 or A3 showed signs of the autophagy pathway. The nucleus to cytoplasm ratios of TEM micrographs (n=12 per treatment) of the cells from each of the treatment groups demonstrated that the increase in the nucleus observed with RV alone was decreased with the addition of the arachidins to a similar size of the untreated or arachidin alone cells. qRT-PCR analysis of gene transcripts, that are important in regulations of the apoptosis and autophagy pathways, validated the TEM observation. The up regulation of the apoptosis transcripts were down regulated with the addition of the arachidins, whereas only beclin-1 and bcl-2 where regulated like the apoptosis transcripts. This pattern of regulation implies a cross talk between the two pathways. Our data suggest a mechanism of action of two natural small molecules showing anti-RV activity that implies potential therapeutic applications.

References


Funding

This work was supported by 1) The Texas A&M Health Science Center / Texas A&M University System Emerging Infectious Diseases Initiative (EID; NIH Grant 1 P01 AI129103-01A1); 2) USDA-NIFA GRANT 2014-68003-24359 and USDA-NIFA Awards 68002979 and 68003242; and 3) The NSF-EPSCoR (grant# EPS-0701890; Center for Plant-Powered Production-P3).

Conclusion

From the data collected, the viability of the cells was not impacted with the introduction of A1 or A3, therefore the arachidins do not adversely affect the cells. However, the amount of infectious virus particles produced when treated with A1 and A3 was decreased by approximately one hundred fold and were both statistically significant (p=2.4E-5). Also, TRPS analysis showed a size pattern of distribution of particles that was consistent with the size range of virus particles measured by TEM of the more mature nonenveloped RV (neRV 67-73.5nm) and more immature enveloped RV (eRV 111-117nm). In addition, TRPS analysis of RV only supernatants demonstrated a size population that is consistent with infectious RV, but with a few larger immature RV particles. On the other hand, the arachidin treated cell supernatants show a population of more immature RV sizes. This suggests that the arachidins do impact RV maturation. The TEM images depicted changes of the ultrastructures of RV-infected HT29.f8 cells, and indicates apoptosis while RV-infected cells with the addition of A1 or A3 showed signs of the autophagy pathway. The nucleus to cytoplasm ratios of TEM micrographs (n=12 per treatment) of the cells from each of the treatment groups demonstrated that the increase in the nucleus observed with RV alone was decreased with the addition of the arachidins to a similar size of the untreated or arachidin alone cells. qRT-PCR analysis of gene transcripts, that are important in regulations of the apoptosis and autophagy pathways, validated the TEM observation. The up regulation of the apoptosis transcripts were down regulated with the addition of the arachidins, whereas only beclin-1 and bcl-2 were regulated like the apoptosis transcripts. This pattern of regulation implies a cross talk between the two pathways. Our data suggest a mechanism of action of two natural small molecules showing anti-RV activity that implies potential therapeutic applications.