

Equine arteritis virus cytopathic effect: caspase-dependent cell death as the major consequence observed

Abstract

Equine arteritis virus infection in horse populations could be confirmed by the OIE recommended Virus Neutralization (VN) test and by the gold standard Virus Isolation (VI). These two techniques involve the observation of the cytopathic effect (CPE) of EAV. The characteristic CPE in EAV infections is the cellular lysis. The presence/absence of this CPE in cells in the VI/VN respectively, indicate the positivity of each test.

CPE refers to morphological and molecular changes that where evidence in infect cells after viral infections. Most viral infections eventually result in the death of the host cell by different cellular mechanisms. The causes of death include cell lysis by alterations to the cell's surface membrane and by various modes of programmed cell death such as necrosis, apoptosis, autophagy and others. EAV CPE is always refers as cellular lysis but the mechanism involve in this lytical effect has never specifically determined. So, our objective is to extend the concept of the cytopathic effect of EAV infections. To study the effect of different cell death mechanism in EAV CPE we used different inhibitors. Consequently, we concluded that the most important mechanism of cell death in EAV infections is caspase-dependent cell death.

Keywords: equine arteritis virus, cytopathic effect, caspase-dependent cell death

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Abbreviations: VN, virus neutralization; VI, virus isolation; EAV, equine arteritis virus; CPE, cytopathic effect; MOI, multiplicity of infection; FBS, fetal bovine serum; MEM, minimal essential medium; PI, postinfection

Short Communication

The order Nidovirales comprises the families Arteriviridae, Coronaviridae, Mesoniviridae, and Roniviridae.¹ Equine arteritis virus (EAV) is the prototype member of the family Arteriviridae and it has been grouped solely in the new genus Equarterivirus.² EAV is a small enveloped virus with a 12.7-kb positive-sense single-stranded RNA genome which includes ten functional open reading frames.³ EAV is an important equine respiratory virus causing equine viral arteritis (EVA). The majority of naturally acquired infections due to EAV are subclinical but if clinical signs are present they can vary in range and severity.⁴ Sporadic occurrence of clinical disease are characterized principally by fever, edema, anorexia, urticarial-type skin reaction, conjunctivitis, abortion and rarely, a fulminating pneumonia, enteritis or pneumoenteritis in young foals. A carrier state can occur in a variable percentage of infected stallions, but not in mares, geldings or sexually immature colts. The carrier state in infected stallion occurs for a variable period of time and there is no evidence of intermittence in virus shedding.

Viral infection in horse populations could be confirmed by the OIE recommended Virus Neutralization (VN) test and by the gold standard Virus Isolation (VI). These two techniques involve the observation of the cytopathic effect (CPE) of EAV. The characteristic CPE in EAV infections is the cellular lysis. The presence/absence of this CPE in cells in the VI/VN respectively, indicate the positivity of each test.

CPE refers to morphological and molecular changes that where evidence in infect cells after viral infections. Most viral infections eventually result in the death of the host cell by different cellular mechanisms.⁵ The causes of death include cell lysis by alterations to the cell's surface membrane and by various modes of programmed cell death such as necrosis, apoptosis, autophagy and others. EAV CPE is always refers as cellular lysis but the mechanism involve in this lytical effect has never specifically determined. So, our objective is to extend the concept of the cytopathic effect of EAV infections.

To study the effect of different cell death mechanism in EAV CPE we used different inhibitors. Each inhibitor was pre-incubated in cell monolayer for one hour prior viral infections in order to block a specific cell death pathway. After viral infection the inhibitors were reseeded and maintained until cell harvest. The investigation was done using Vero E6 cell line as a sensitive cell culture for EAV.^{6,7} Monolayers with 80% confluency arranged in 12-well plates were used and infected with the reference EAV strain, Bucyrus (Pubmed Reference: DQ846750) at a multiplicity of infection (MOI) of 1. The monolayers were incubated for one hour at 37°C in an atmosphere with 5% CO₂. Then, the inoculum was removed and maintained in Minimal Essential Medium (MEM) with 2% fetal bovine serum (FBS) and with each inhibitor at same concentration. All samples analyzed in this experiment were harvested at 72h postinfection (pi), which is the standardized time at the maximal CPE evidenced in EAV-infected cells.⁸ Uninfected cultures were used as negative controls. Each of the experiment described was done by triplicate.

The samples were processed for cytometry following the commercial protocol (Thermo Fisher Scientific). After resuspending the cells, they were labeled with 5µl Annexin V-FICT (100µg/

ml, BioLegend) / 1µl Propidium Iodide (50µg/ml, Thermo Fisher Scientific) and subjected to cytometry. After analyzing 10,000 events, the results were plotted using the Flow Jo program. The figures were displayed in different quadrants as follow: the lower left quadrants (Q4) refer to intact cells (Annexin V⁻, PI⁻); the lower right quadrants (Q3) represent early apoptotic cells (Annexin V⁺, PI⁻) and the upper right quadrants (Q2) refer to late apoptotic and/or necrotic cells (Annexin V⁺, PI⁺). The control monolayers of uninfected Vero E6 cells showed a percentage of 81.9% live cells (Figure 1A, Q4 quadrant), that was reduced dramatically to 37.5% in EAV-infected Vero E6 cells (Figure 1B, Q4 quadrant).

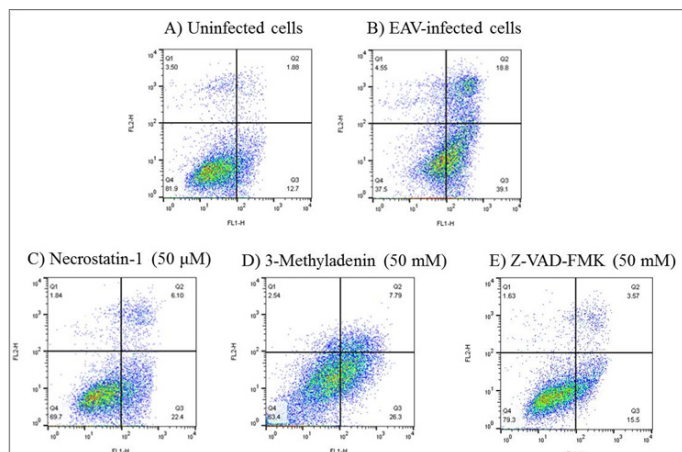


Figure 1 Flow cytometry of control and treated cell monolayers with each inhibitor. Q4 quadrant showed the percentage of cell survival in each experiment.

- A) uninfected cells:81.9%;
 B) EAV-infected cells:37.5%;
 C) Necrostatin-1 (50µM):69.7%;
 D) 3-Methyladenin (50mM):63.4%.
 E) ZVA-D-FMK (50mM):79.3%.

To analyze the necrosis involve in the EAV CPE we used 1µl Necrostatin-1(50µM, Abcam, Cambridge, MA), a recognized inhibitor of RIPK1 kinase in the necrosis process.⁹ The cytometry showed in this case a percentage of cell survival of 69.7% (Figure 1C, Q4 quadrants). As it was not reached the percentage of 81.9% of cell survival as in the uninfected culture it was assumed that should exists other form of cell death in EAV-infected cells. To analyze the autophagy involve in the EAV CPE we used 1µl of autophagy inhibitor 3-Methyladenin (50 mM, Sigma-Aldrich). 3-Methyladenin inhibits autophagy by blocking autophagosome formation via the inhibition of type III phosphatidylinositol 3-kinases (PI-3K).¹⁰ In this case, we obtained a percentage of 63.4% of survival cells (Figure 1D, Q4 quadrants) indicating that the autophagy mechanism was not the main mechanism in cell death in EAV infection.

Finally, we studied the importance of caspase-dependent cell death in the CPE of EAV-infected cells. For this purpose, we used as a pan-caspase inhibitor 1µl Z-VAD-FMK (50mM, Abcam, Cambridge, MA). This is a cell-permeant pan-caspase inhibitor that irreversibly binds to the catalytic site of caspases and can inhibit induction of all kinds of caspase-dependent cell death.¹¹ When this cell death pathway

was inhibited in infected cultures we observed the percentage of cell survival (79.3%) (Figure 1E, Q4 quadrants) reached similar values as uninfected Vero E6 (81.9%). Consequently, thus indicate the crucial importance of caspase-dependent cell death in the CPE of EAV infection. Several scientific papers reported the relations between EAV and cellular apoptosis.^{7,12-15} Nevertheless, this mechanism was never associated with the CPE of EAV infection in cell cultures. Consequently, in this easy set of experiments we concluded that the most important mechanism of cell death in EAV infections is caspase-dependent cell death or saying in other words, EAV CPE is due to a caspase-dependent death.

Conclusion

We redefining equine arteritis virus cytopathic effect and point the caspase-dependent mechanism as the major consequence observed in cell cultures. The activity of individual and specific caspases must be analyzed to confirm the particular caspase/s that mediated the cell death mechanism particularly involved.

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Conflict of interest

The author declares no conflict of interest.

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