Fish & Shellfish Immunology 51 (2016) 41–45

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Short communication

Effects of a broad-spectrum caspase inhibitor, Z-VAD(OMe)-FMK, on viral hemorrhagic septicemia virus (VHSV) infection-mediated apoptosis and viral replication

Min Sun Kim, Ji Ae Lee, Ki Hong Kim*

Department of Aquatic Life Medicine, Pukyong National University, Busan 608-737, South Korea

ARTICLE INFO

Article history:
Received 27 November 2015
Received in revised form
30 December 2015
Accepted 16 February 2016
Available online 17 February 2016

Keywords:
VHSV
Apoptosis
Caspase inhibitor
Viral titer

ABSTRACT

In the development of inactivated or attenuated viral vaccines for cultured fish, viral titers harvested from the cultured cells would be the most important factor for the determination of vaccine's cost effectiveness. In this study, we hypothesized that the lengthening of cell survival time by the inhibition of apoptosis can lead to an increase of the final titer of viral hemorrhagic septicemia virus (VHSV). To test the hypothesis, we investigated the effects of a broad-spectrum caspase inhibitor, Z-VAD(OMe)-FMK, on VHSV infection-mediated apoptosis in *Epithelioma papulosum cyprini* (EPC) cells and on the VHSV titers. VHSV infection induced the DNA laddering in EPC cells, and the progression of DNA fragmentation was in proportion to the CPE extension. The progression of DNA fragmentation in EPC cells infected with VHSV was clearly inhibited by exposure to Z-VAD(OMe)-FMK, and the inhibition was intensified according to the increase of the inhibitor concentration. These results confirmed the previous reports that the death of host cells by VHSV infection is through apoptosis. Cells infected with a recombinant VHSV, rVHSV-ΔNV-eGFP, that was generated from our previous study by replacement of the NV gene ORF with the enhanced green fluorescent protein (eGFP) gene ORF, showed earlier and more distinct DNA fragmentations compared to the cells infected with wild-type VHSV, suggesting the inhibitory role of the NV protein in VHSV-mediated apoptosis that was previously reported. The final viral titers in the supernatant isolated from Z-VAD(OMe)-FMK treated cells after showing an extensive CPE were significantly higher than the viral titers from cells infected with virus alone, indicating that the delay of apoptosis by Z-VAD(OMe)-FMK extended the survival time of EPC cells, which lengthen the time for VHSV replication in the cells. In conclusion, Z-VAD(OMe)-FMK-mediated inhibition of apoptosis significantly increased the final titers of both wild-type VHSV and rVHSV-ΔNV-eGFP, indicating that apoptosis inhibition can be a way to get higher titers of VHSV.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Apoptosis is a representative response of hosts against viral infections. As viruses are wholly dependent upon the host cell's nurturing machinery for their replication, apoptosis of cells before viral replication can lead to a catastrophe for viruses. However, many viruses can utilize apoptosis as a tool for the dissemination of viruses from infected cells. Thus, the time of apoptosis initiation in cells infected with viruses can be a critical factor that determines whether the apoptosis is beneficial or harmful to hosts [1–3].

Viral hemorrhagic septicemia virus (VHSV) is an enveloped, negative-sense RNA virus belonging to the genus *Novirhabdovirus* of the family Rhabdoviridae, and has been a cause of mass mortality of cultured fish worldwide [4,5]. Members of Rhabdoviridae such as vesicular stomatitis virus (VSV), rabies virus (RAV) in mammals, and spring viraemia carp virus (SVCV), turbot rhabdovirus (SMRV), infectious hematopoietic necrosis virus (IHNV), VHSV in fish have been known to induce apoptotic cell death [6–11]. Ammayappan and Vakharia [12] recently reported that NV protein of VHSV and IHNV suppressed apoptosis of infected cells in an early infection phase. These reports suggest that rhabdoviruses can utilize or regulate host cell apoptosis for their dissemination.

Outbreaks of VHSV disease in olive flounder (*Paralichthys olivaceus*) farms have been a major problem to increase the

* Corresponding author.
E-mail address: khkim@pknu.ac.kr (K.H. Kim).

http://dx.doi.org/10.1016/j.fsi.2016.02.021
1050-4648/© 2016 Elsevier Ltd. All rights reserved.

productivity of the farms in Korea [13]. Recently, vaccines based on the inactivated VHSV or the attenuated VHSV have been experimentally tried in olive flounder [14,15]. However, as cost-effectiveness is essential in the development of fish vaccine, the viral titer harvested from the cultured cells is the most important factor for the determination of vaccine practicality. Although apoptosis can influence on the viral titers, utilization of the apoptosis to get higher viral titers has not been investigated in VHSV. In this study, we hypothesized that the lengthening of cell survival time by the inhibition of apoptosis can lead to an increase of the final titer of VHSV.

Caspases play a critical role in the initiation and execution of apoptosis. The initiator caspases such as caspase-8 and -9 that are activated through the extrinsic or intrinsic pathways of apoptosis cleave and activate the executioner caspases (caspase −3, −6, and −7), leading to the degradation of essential proteins and DNA [16,17]. Apoptosis can be suppressed by caspase inhibitors. A synthetic cell-permeable tripeptides, *N*-Benzyloxycarbonyl-Val-Ala-Asp(OMe)-fluoromethyl ketone (Z-VAD(OMe)-FMK), acts as a pseudo-substrate for caspases by binding to the catalytic site irreversibly, by which apoptosis is inhibited [18]. In this study, we investigated the effects of Z-VAD(OMe)-FMK on VHSV infection-mediated apoptosis in *Epithelioma papulosum cyprini* (EPC) cells and on the VHSV titers.

2. Materials and methods

2.1. Cells and viruses

E. papulosum cyprini (EPC) cells were grown in Leibovitz medium (L-15, Sigma) supplemented with 10% fetal bovine serum (FBS, BI) and MycoZap Antibiotics (2 μl/ml; Lonza). The VHSV KJ2008 used in this study was isolated from a diseased olive flounder (*P. olivaceus*) and the genotype was IVa based on the G gene sequence [19]. A recombinant VHSV, rVHSV-ΔNV-eGFP, used in this study was generated previously by replacement of the NV gene ORF with eGFP gene ORF [19]. The viruses were propagated in monolayer of EPC cells at 15 °C in the presence of 2% FBS. When an extensive cytopathic effect (CPE) was observed, the cells were submitted to two cycles of freeze-thawing and centrifuged at 4000 g for 10 min to collect supernatant.

2.2. DNA fragmentation of EPC cells by VHSV infection

The progression of EPC cell's apoptosis caused by VHSV infection was measured by DNA fragmentation that is a hall mark of apoptosis. The cultured EPC cells (1×10^6 cells/well) in L-15 supplemented with 2% FBS and MycoZap antibiotics at 15 °C were infected with VHSV KJ2008 at a multiplicity of infection (MOI) of 0.1. At 6, 12, 18, 24, 36, 42, 48, 54, 60, 66, and 72 h post-infection, cells were washed twice with phosphate buffered saline (PBS) and the genomic DNA was isolated using Exgene™ Clinic SV kit (GeneAll Biotechnology, Korea) according to manufacturer's instructions. The isolated DNA samples were electrophoresed on 1.5% agarose gels.

2.3. Effect of an apoptosis inhibitor on VHSV-mediated apoptosis and VHSV titer

A broad-spectrum caspase inhibitor, Z-VAD(OMe)-FMK (Santa Cruz), was used to inhibit apoptosis caused by VHSV infection. The inhibitor was dissolved using dimethyl sulfoxide (DMSO, Sigma) at a 20 mM concentration (stock solution), and then diluted with L-15 medium to get working solutions. EPC cells (1×10^6 cells/well) were exposed to 10, 30, or 50 μM of the inhibitor for 2 h and then infected with either wild-type VHSV or rVHSV-ΔNV-eGFP at a MOI of 0.1. At 24, 36, 48, 60, and 72 h post-infection, supernatant was collected for viral titration, and cells were washed with PBS and then DNA was isolated for DNA fragmentation assay. In addition, to measure the final titers of the viruses, supernatant was collected after the observation of an extensive CPE in each group of cells. The experiments were conducted in triplicate. The viral titers were determined by the plaque assay according to Burke and Mulcahy [20]. Briefly, EPC cells were inoculated with each viral sample that were serially diluted from 10^{-4} to 10^{-6}. After 2 h of incubation at 15 °C, the cells were overlaid with plaquing medium (0.7% agarose in L-15 containing 2% FBS and antibiotics). At 7 days post-infection, the cells were fixed by 10% formalin and stained with 3% crystal violet for 30 min at room temperature. After rinsing of the cells with distilled water, the plaque-forming units (PFU) were counted.

2.4. Statistical analysis

The statistical significance of the data was determined by using analysis of variance (ANOVA); followed by Tukey HSD post-hoc test (SPSS for Windows), and $P < 0.05$ was considered statistically significant.

3. Results

3.1. VHSV-mediated DNA fragmentation

The progression of DNA fragmentation in EPC cells infected with wild-type VHSV at a MOI of 0.1 is shown in Fig. 1. DNA fragmentation was not observed until 24 h post-infection, then, became gradually distinct to 72 h post-infection. The genomic DNA isolated from cells infected with VHSV showed a 200 bp ladder pattern, but DNA from the mock-infected control cells showed no DNA fragmentation (data not shown).

3.2. Effect of Z-VAD(OMe)-FMK on VHSV-mediated CPE

Cells treated with Z-VAD(OMe)-FMK showed a weaker CPE than control cells by infection with either wild-type VHSV or rVHSV-ΔNV-eGFP (Fig. 2A,B). In the cells not treated with Z-VAD(OMe)-FMK, the CPE progression by rVHSV-ΔNV-eGFP was slower than that by wild-type VHSV. At 72 h post-infection, cells infected with wild-type VHSV or rVHSV-ΔNV-eGFP without the caspase inhibitor showed evident CPE throughout the entire monolayer, but a large proportion of the cells treated with the caspase inhibitor before viral infections showed an intact cell shape, and the CPE was weaker in accordance with the increase of Z-VAD(OMe)-FMK concentrations (Fig. 2).

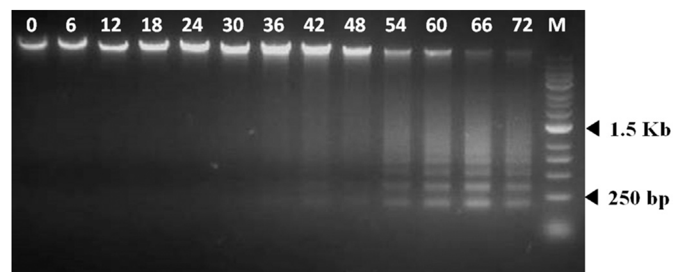

Fig. 1. DNA fragmentation of Epithelioma papulosum cyprini (EPC) cells by infection with viral hemorrhagic septicemia virus (VHSV) at a MOI of 0.1. At 0, 6, 12, 18, 24, 36, 42, 48, 54, 60, 66 and 72 h post-infection, genomic DNA was isolated and electrophoresed through 1.5% agarose. M, 1 Kb DNA ladder (GeneAll).

Download English Version:

https://daneshyari.com/en/article/2430747

Download Persian Version:

https://daneshyari.com/article/2430747

Daneshyari.com