

Inhibition of EMCV Replication in BHK-21 Cells by Regulating ROS Microenvironment Based on Novel Hydrogen Sulfide Sustained-release Nanomaterials

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Abstract

Background: Encephalomyocarditis virus (EMCV) infection often induces excessive reactive oxygen species (ROS) production in host cells. (ROS), destroy redox homeostasis and promote viral replication. Mitochondria are cell energy centers and immune signal hubs, and their dysfunction is closely related to viral pathogenicity. Hydrogen sulfide (H₂S), as an endogenous gas signal molecule, has significant antioxidant and mitochondrial protection potential, but its short half-life and lack of targeting limit its clinical application. Recent studies have shown that the use of nanomaterials to achieve precise delivery of H₂S and microenvironment regulation of ROS has become a new strategy against viral infection. The purpose of this study was to construct a ROS-responsive H₂S sustained-release nanosystem and to explore its synergistic antiviral effect and mechanism on EMCV-infected BHK-21 cells. **Methods :** H₂S donor nanomaterials and superoxide dismutases with ROS response characteristics were prepared. (SOD) nanozyme. The EMCV infected BHK-21 cell model was established, and the biocompatibility was evaluated by CCK-8 method. The viral load was determined by qRT-PCR and plaque assay. The intracellular ROS level and mitochondrial membrane potential were detected by fluorescent probe. The expression of key proteins in NRF2 antioxidant pathway and type I interferon signaling axis was analyzed by Western Blot. **Results :** Experiments showed that the nanomaterial effectively removed the peak of intracellular ROS in the early stage of infection, maintained the integrity of mitochondrial function, and blocked the path of virus using oxidative stress signal to promote replication. The combined treatment group showed a significant synergistic effect, and the virus titer was reduced by more than one order of magnitude compared with the control group. Mechanistically, the precise release of H₂S enhances NRF2-mediated antioxidant defense and up-regulates the expression of interferon-stimulated genes (ISGs), reshaping the host microenvironment that is not conducive to virus propagation. **Conclusion :** This study confirmed that the H₂S / ROS synergistic regulation strategy based on new materials can effectively inhibit EMCV replication by reshaping the host redox microenvironment. This strategy avoids the shortcomings of traditional direct antiviral drugs that are prone to drug resistance, and provides a new paradigm for the development of broad-spectrum nanomedicines for positive-strand RNA viruses. In the future, its efficacy and safety in vivo models will be further evaluated to promote clinical transformation.

Keywords

Encephalomyocarditis Virus (EMCV), BHK-21 Cells, Hydrogen Sulfide (H₂S), Reactive Oxygen Species (ROS), Nanomaterials, Oxidative Stress