Responses of Neural Cells (U87) to HCoV-229E and HCoV-OC43 Infections

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Abstract: Respiratory viruses, such as Coronaviruses (CoVs), can be neuroinvasive and exacerbate neurological pathologies via their capacity for direct viral replication and/or by inducing excessive host immune responses in the Central Nervous System (CNS). HCoV-229E is a primary COVID-19 and HCoV-OC43 is a beta COVID-19 belonging to the same genus as SARS-CoV-2 and both can cause acute infections in glioblastoma, neuroblastoma, and germline cells. These viruses tend to exhibit different infectivity mechanisms and HCoV-OC43 tends to be more toxic than HCoV-229E for CNS cells. To gain an in-depth understanding of how respiratory viruses, such as SARS-CoV, infect nerve cells (U87) and promote inflammation and determine the different mechanisms underlying HCoV-229E and HCoV-OC43 infection, we evaluated Lactic Acid Dehydrogenase (LDH) activity, cell viability, and IL-8 absorptions. HCoV-OC43 tended to show greater cytotoxicity against U87 nerve cells than HCoV-229E. Further study into the connection between the HCoV-229E and HCoV-OC43 viruses and brain cell reactions will be supported by our results.

Keywords: Cell Viability, Cytotoxicity, HCoV-OC43, HCoV-229E, U87 Cells

Introduction

Human Coronaviruses (HCoVs) are a collection of single-stranded RNA viruses associated with respiratory, intestinal, and neurological diseases in humans and livestock (Lee et al., 2010) in humans, they mainly cause respiratory infections. HCoVs are the largest known RNA viruses and are divided into four types: Alpha, beta, gamma, and delta (Acosta et al., 2020; Yin and Wunderink, 2018). To date, six HCoVs with various respiratory manifestations, such as pneumonia and bronchitis (Yin and Wunderink, 2018), have been identified, including Human Coronavirus (HCoV)-NL63, and HCoV-229E, β-CoV-HCoV-OC43, HCoV-HKU1, Severe Acute Respiratory Syndrome-CoV (SARS-CoV2) and Middle East Respiratory Syndrome-Related Coronavirus (MERS-CoV). HCoV-229E and HCoV-OC43 were characterized by long-term cultures of patients diagnosed with upper respiratory disease in the 1960s, at which point they were considered new pathogens that resembled a temporary common cold (Huang et al., 2020).

Hematogenous dissemination and neuronal retrograde dissemination are the two different ways that infections can reach the Central Nervous System (CNS) (Desforges et al., 2014). Hematophilic proliferation includes the occurrence of a specific infection in the bloodstream, whereas retrograde virus-related proliferation happens when the infection contaminates neurons in the periphery and then uses a transport mechanism inside the cell to contact the CNS (Berth et al., 2009). Viruses such as HCoV-229E, HCoV-OC43, and SARS-CoV can use these peripheral access pathways to facilitate their neuroinvasive properties (Desforges et al., 2014).

HCoV-229E and HCoV-OC43 have different infectivity profiles toward CNS cells. Studies of susceptible mouse models have shown that HCoV-OC43 primarily enters the Central Nervous System (CNS) via the olfactory pathway and nerve cells to nerve cells transfer, focusing on the piriform cortex, the brainstem, and the spinal cord (Huang et al., 2020). However, they can both cause acute infections in cell lines derived from neuroblastoma, glioblastoma, and sex cells (Huang et al., 2020).

The literature on CoVs has generally focused on respiratory manifestations. However, in recent decades, several studies have shown that respiratory viruses, including HCoVs, have neuroinvasive
potential (Morris and Zohrabian, 2020), and several HCoVs have now been identified as potential neuroviruses in both children and adults. However, no clear link between CoVs and human neuropathology has yet been identified. Furthermore, HCoV-229E, HCoV-OC43, and SARS-CoVs all have neuroinvasive and neurotropic properties (Desforges et al., 2014). It is also called that HCoV infections can spread from the lungs and cause neurological complications (Alam et al., 2020).

HCoV-229E and HCoV-OC43 reportedly infect macrophages and HCoV-229E can infect human brain endothelial cells, providing an alternative pathway for CNS infiltration (Arbour et al., 2000). Accumulating clinical evidence suggests that these opportunistic pathogens can overcome immune responses and affect non-respiratory organs, including the CNS (Correa et al., 2020). To better understand the potential for CoVs to function as neuroviruses, their potential neurovirulence and neuroinvasion characteristics must be investigated (Arbour et al., 2000).

Thus, the objective of this research was to assess the feasibility of U87 cells, a human glioblastoma cell line usually used in brain cancer research (Clark et al., 2010), exposed to different concentrations of HCoV-OC43 and HCoV-229E and analyze changes in cytotoxicity and cytokine levels. The results provide a basic reference for the effects of HCoV-OC43 and HCoV-229E on glial cells.

Materials and Methods

Virus Culture

HCoV-229E and HCoV-OC43 strains were purchased from the Korea Bank for Pathogenic Viruses (https://www.kbpv.re.kr/). Viruses were propagated in MRC-5 cells from the Korean Cell Line Bank (Seoul, Republic of Korea) to prepare stock viruses. Monolayers of MRC-5 cells were infected with each virus and left for 24 h to allow adsorption. For viral adsorption, MRC-5 cells were seeded in 10 cm dishes with 15 μg of either HCoV-229E or HCoV-OC43. Cell supernatants were collected after another 48 h. Cell debris was cleared by extractor at 3000 rpm for 6 min and the cells are then aliquoted and deposited at -80°C.

Cell Culture

U87, a person’s brain glioblastoma epithelial cell line, was acquired from the Korean Cell Line Bank. The cells (1x10^4 cells/well) were seeded in 96-well plates in the least vital Eagle’s material (M4655; Sigma Gangnam-gu, Republic of Korea) under a moistened atmosphere (5% CO2) at 37°C for 24 h. The total medium is increased with 10% Fetal Bovine Serum (FBS; Corning, Glendale, CA, USA) and 100 units/mL Gibco™ penicillin-streptomycin (Thermo Fisher Scientific, Seoul, Republic of Korea).

Cell Exposure to Virus

Cell monolayers (1x10^4 cells/well) were infested with HCoV-OC43 or HCoV-229E. U87 was used at a Multiplicity of Infection (MOI) of 0.1, 1.0, or 10.0 in the presence of Trypsin-EDTA (0.25%) (Thermo Fisher Scientific) Cultures were raised at 37°C and 5% CO2 for 24 h post-infection (hpi). The control group consisted of non-infected cells cultured in DMEM alone.

Cell Viability

The effects of HCoV-OC43 and HCoV-229E on cell viability were defined using a Quanti-MAX™ Water-Soluble Tetrazolium (WST)-8 Cell Viability Assay Kit (Biomax, Seoul, Republic of Korea). Briefly, 10 μL of WST was increased in each well and the wells were nurtured for 0.5 h. The absorbances at 450 nm were then measured using a microplate reader (Biomax, Seoul, and the Republic of Korea).

Lactate Dehydrogenase Release

A Quanti-LDHTM PLUS Cytotoxicity Assay Kit (Biomax) was used to track the discharge of Lactate Dehydrogenase (LDH) from cells into the culture medium. HCoV-OC43 and HCoV-229E's cytotoxicities were assessed by calculating the LDH level using a colorimetric test. From each well, aliquots (100 L) of the cell culture media were taken and put in fresh microtiter plates. Each well received 100 L of LDH and the plates underwent a 0.5 h incubation period at 37°C and 5% CO2. Then, utilizing a Flex Station 3 Multi-Mode Microplate Reader (Molecular Devices, San Jose, CA, USA), sample absorbances were determined at 490 nm. Each experiment was carried out four times. Each experiment was carried out four times. Cytotoxicity was measured in relation to the baseline LDH level produced by untreated control cells cultured in FBS-free media. The experiment was repeated three times and the percentage of LDH discharge by cells that had been subjected to HCoV-OC43 and HCoV-229E was calculated.

Interleukin-8 (IL-8) Concentrations

An ELISA MAXTM Deluxe Set Human IL-8 Kit (BioLegend, San Diego, CA, USA) was used to measure the levels of IL-8. The collected antibody was dissolved in 1 Coating Buffer A the day before the ELISA and 100 L of this mixture was applied to each well of a 96-well plate. After that, the plate was closed and maintained at 2-8°C for 18 h. Then, 100 L each of the BioLegend TMB substrate solution and the BioLegend diluted Avidin-HRP solution were mixed. Utilizing a FlexStation 3 Multi-Mode Microplate Reader (Molecular Devices), the absorption at 490 nm was determined once materials had been incubated at 37°C for 15 min.
Statistical Analysis

One-way analysis of variance was utilized in the statistical studies utilizing Prism6 (Version 6.02, March 11, 2013). A post-hoc test was employed as well and the outcomes are shown as the mean and standard error of the mean. Statistical significance was defined as a p-value of 0.05.

Results

U87 Cell Viability

The viabilities of cells exposed to HCoV-229E at MOI 0.1, MOI 1, and MOI 10 were 83.8±0.03, 77.8±0.06 and 78.1±0.02%, respectively, at 0 h and 91.0±0.08, 70.0±0.04 and 76.1±0.07%, respectively, at 24 h (Fig. 1). The feasibility of U87 cells improved over a period after infection with HCoV-229E at MOI 0.1 but decreased after infection at MOI 1 and MOI 10.

The viabilities of cells exposed to HCoV-OC43 at MOI 0.1, MOI 1, and MOI 10 were 75.9±0.01, 81.8±0.02 and 84.7±0.0%, respectively, at 0 h and 96.2±0.04, 85.5±0.03 and 64.6±0.04%, respectively, at 24 h (Fig. 1). The feasibility of U87 cells improved after infection with HCoV-OC43 at MOI 0.1 and MOI 1 but decreased at MOI 10.

LDH Activity in U87 Cells

LDH activity in U87 cells was intended to link the absorbance of the medium from HCoV-229E-exposed cells and unexposed cells. LDH activity levels after exposure to HCoV-229E at MOI 0.1, MOI 1, and MOI 10 were 91.9±0.04, 104.4±0.05 and 251.2±0.05%, respectively, after 0 h and 99.4±0.05, 101.7±0.06 and 173.4±0.0%, respectively, after 24 h (Fig. 2). LDH activity in U87 cells improved after contact to HCoV-229E at MOI 0.1 but decreased at MOI 1 and MOI 10. Additionally, LDH activity in U87 cells decreased significantly over time after exposure to HCoV-229E at MOI 10.

These results indicate that higher MOIs affected LDH activity at the investigational time points evaluated in this research. After exposing U87 cells to HCoV-OC43 at MOI 0.1, MOI 1, and MOI 10, LDH activities were 102.9±0.04, 196.0±0.01 and 501.0±0.05%, respectively, after 0 h and 104.1±0.06, 163.9±0.03 and 330.3±0.01%, respectively, after 24 h (Fig. 2). The LDH activity of U87 cells improved after contact to HCoV-OC43 at MOI 0.1, whereas it decreased at MOI 1 and MOI 10. Additionally, LDH activity decreased significantly over time after exposure to HCoV-229E at MOI 1 and MOI 10.

IL-8 Levels in U87 Cells

The amount of IL-8 unrestricted from U87 cells was intended by linking the absorbance of the material from HCoV-229E-exposed cells and unexposed cells. IL-8 levels measured after exposure to HCoV-229E at MOI 0.1, MOI 1, and MOI 10 were 242.0, 411.7, and 1745.2 pg/mL, after 0 h and 1100.7, 2061.2 and 4041.7 pg/mL after 24 h, respectively (Fig. 3). The amount of IL-8 in U87 cells generally improved after contact to HCoV-OC43 at MOI 1 and MOI 10. Additionally, the amount of IL-8 augmented considerably after exposure to MOI 10 HCoV-229E.

After exposing U87 cells to HCoV-OC43 at MOI 1, MOI 1, and MOI 0, IL-8 levels were 294.7, 3215.2, and 10343.2 pg/mL after 0 h and 895.7, 4860.2 and 10828.2 pg/mL after 24 h, respectively (Fig. 3). These outcomes propose that IL-8 degree in U87 cells growth proportionally with the concentrations of HCoV-229E and HCoV-OC43.
Discussion

We assessed LDH action, cell feasibility, and IL-8 concentrations to define the effects of HCoV-229E and HCoV-OC43 exposure on glial cells. Neurons were treated with three different virus concentrations, revealing that higher concentrations of the target viruses led to increased toxicity and decreased viability. In agreement with these results, low concentrations of the viruses did not affect cell survival.

Brison et al. (2014) showed that HCoV-OC43 infection can lead to nerve loss (neural degeneration), with significant increases in extracellular glutamic acid levels and LDH release.

LDH discharge is a sign of necrosis (Soletti et al., 2010; Eslaminejad et al., 2022). WST colorimetry is commonly utilized to assess cell feasibility by computing the action of intracellular mitochondrial enzymes, and signs of intracellular mitochondrial purpose (Soletti et al., 2010; Eslaminejad et al., 2022).

The LDH activity and IL-8 concentration of U87 cells were highest under infection with HCoV-229E at an MOI of 0.1 and with HCoV-OC43 at an MOI of 0.1. In addition, IL-8 levels immediately responded in a dose-dependent manner after infection with HCoV-OC43 in glial cells; however, there were no significant differences in IL-8 levels after 24 h. In contrast, HCoV-229E, an alpha coronavirus, caused a large increase in IL-8 24 h after viral infection in glial cells.

High IL-8 levels in cerebrospinal fluid were observed by Correia et al. (2020). Li et al. (2017) showed that IL-6 and IL-8 were deposited in the cerebrospinal fluid of patients with CoV-CNS infections. According to these findings, CoV-CNS illnesses are widespread, and a variety of cytokines are implicated in the initial immunological reaction to the illness, resulting in immunodeficiencies in the brain (Correia et al., 2020).

IL-8 is a chemokine that acts as a strong chemical cause for polymorphic nuclear cells and lymphocytes and is connected with blood-brain barrier collapse (Correia et al., 2020). Chemokines, cytokines, and other inflammatory signals can also trigger massive neuroinflammatory responses (Abdelaziz and Waffa, 2020). Reactive neuroglial cells define a selection of cytokines and chemokines (Anderson et al., 2016; Liddelow et al., 2017), causing the blood-brain barrier to collapse and subsequently allowing auto-reactive lymphocytes to infiltrate the CNS (Binder, 2018). Brison et al. (2014) showed that HCoVs can infect and replicate in U87 cells, resulting in the generation of inflammatory mediators (Edwards et al., 2000). Early growth in the provocative cytokine reaction may thus support preparing host cells for pathogen removal (Jakhmola and Jha, 2021).

A glial cell helps to maintain brain homeostasis and is an important controller of neuroinflammation in the CNS (Anderson et al., 2016; Liddelow et al., 2017). Infested glial cells will also directly or indirectly activate apoptosis in adjacent glial cells (Jakhmola and Jha, 2021).

Several respiratory viruses, including HCoVs, have neuroinvasive potential and exacerbate neurological pathologies as a result of direct viral replication or by activating excessive active host immune responses in the CNS (Morris and Zohrabian, 2020). HCoV-229E and HCoV-NC83 are alpha COVID-19, while all other known HCoVs are beta coronaviruses (Alam et al., 2020).

Interestingly, HCoV-OC43, a beta coronavirus in the same genus as SARS-CoV-2, was found to be more toxic than HCoV-229E to neural cells. High MOI conditions induced rapid apoptosis, making it difficult to include a wide range of MOIs and therefore limiting this study to relatively low values. We did not determine the relative frequencies of apoptosis and necrosis; apoptotic factors were not assessed, representing a limitation of this study. To clearly reveal the relationship between the HCoV-229E and HCoV-OC43 viruses and the neuronal response, further studies using a wider range of MOIs are needed. Other viruses, such as SARS-CoV-2 (the causative cause of the COVID-19 epidemic), should also be included. We will confirm this relationship with a follow-up study and evaluate the effects of the inhibition of apoptosis on cell survival under a high MOI. The results of this study improve our understanding of the relationship between the HCoV-229E and HCoV-OC43 viruses and neural cell responses and the mechanism by which respiratory viruses, such as SARS-CoV, infect neural cells to promote inflammation. Furthermore, our outcomes offer a foundation for an upcoming study on the cause of HCoV-229E and HCoV-OC43 viruses on cellular pathology.

Conclusion

Our results show that inflammatory cytokine levels tend to increase proportionally with the viral concentration in the early stages of infection. In the case of HCoV-229E infection, inflammatory cytokine levels produced by neural cells more than doubled 24 h after infection. The results of this study improve our understanding of the relationship between HCoV-229E and HCoV-OC43 and neural cell responses. Our results thus serve as a foundation for further investigation into the impact of HCoV-229E and HCoV-OC43 on inflammation and the viability of cells.

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Ethics

This investigation was carried out in line with the Declaration of Helsinki’s fundamental principles and was given approval by Dankook University’s Institutional Review Board (Republic of Korea) (No. 2022-08-020).

Competing Interests

The researchers state that they have no opposing interests.

Eun Ju Oh and Jae Kyung Kim: Made substantial contributions to the conception and designed of the study.

Jae-Sik Jeon and Qian-Wen Wang: Made substantial contributions and acquisition and analysis of the data.

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Author’s Contributions

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