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Novel antiviral effect of lithium chloride on mammalian orthoreoviruses in vitro

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ABSTRACT

Reovirus not only causes considerable economic loss in the swine industry of the United States and other countries, but also threatens the public health due to its zoonotic potential. According to previous reports, LiCl has antiviral activity against a number of viruses. The inhibitory effects of LiCl on reovirus life cycle in Vero cells were evaluated. The unpaired t-test and one-way ANOVA were used to analyze the differences between experimental groups. We first found that LiCl treatment significantly inhibited reovirus replication in a dose-dependent manner. Furthermore, we found that this antiviral activity of LiCl targets the early stage of viral replication. LiCl could be a potential drug against reovirus infection.

1. Introduction

The family of Reovirus consists of 15 genera of non-enveloped double-stranded RNA (dsRNA) viruses [1]. Mammalian orthoreoviruses (MRVs) have been isolated from various species, including humans, civet cats, pigs, and bats [2–4], and can be divided into three major serotypes: type 1 Lang (T1L), type 2 Jones (T2J), and type 3 Dearing (T3D) [5–7]. The MRV genome is composed of 10 segments, and divided into three classes: 3 large segments(L1, L2 and L3), 3 medium segments (M1, M2 and M3), and 4 small segments (S1, S2, S3 and S4) [8–10].

MRV was first isolated from children in 1951 and is rarely pathogenic in adults [3]. More often, MRVs cause enteritis, pneumonia, or encephalitis in swine, and have been isolated across the world, including China, South Korea, and the United States [11–15]. However, recent studies have demonstrated that reoviruses from wild animals can cause acute and severe clinical diseases in humans [16–19], raising great concerns about their potential zoonotic property. Furthermore, the zoonotic potential of MRV serotype 3 has been demonstrated [19–21], and reovirus might be association with SARS infection [22,23]. Therefore, the treatment strategy of MRV warrants further investigation.

A series of studies have showed LiCl has antiviral activities on herpes simplex virus, avian infectious brhonchitis virus, transmissible gastroenteritis virus, pseudorabies herpesvirus, type II porcine reproductive and respiratory syndrome virus, and porcine parvovirus [24–31], which indicated the potential of LiCl as a broad-spectrum antiviral drug. Here we report the antiviral activity of LiCl on an MRV isolate from porcine *in vitro*.

2. Results

2.1. Concentrations of LiCl

To investigate the cytotoxicity of LiCl on Vero cells, the cytotoxicity assays were performed according the instructions of the manufacturer of the Cell Counting kit-8(CCK8) (Donjindo, Japan). The relative cell viabilities were above 80% after treatment with LiCl

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at concentrations of 10, 20, 40, and 60 mM, respectively, compared with those under 50% at LiCl concentrations of 100 mM and above (Fig. 1). The 50% cytotoxi cconcentrations (CC_{50}) of LiCl was 99.94 mM. There are no difference in cell morphology was observed (data not shown) with 10–60 mM of LiCl treatment. And the cells were above 80% cytostatic concentration. Therefore, 10–60 mM LiCl were chosen for antiviral assays.

2.2. LiCl inhibited reovirus infection

Vero cells treated with a series of concentrations (0, 10, 20, 40, 60 mM) of LiCl were infected with reovirus. The antiviral effects were determined by the 50% tissue culture infected dose ($TCID_{50}$), real-time quantitative RT-PCR (RT-qPCR) and indirect immunofluorescence assay (IFA). The mean viral titers ($TCID_{50}$/ml) in 0, 10, 20, 40 and 60 mM LiCl-treated cells were 4.77, 4.47, 3.57, 2.39, and 1.41, respectively (Fig. 2A), with the mean relative viral mRNA being 100.00, 97.67, 87.33, 33.33, and 24.33, respectively (Fig. 2B). There were strong fluorescent signals in the group without LiCl treatment, whereas the fluorescent signals declined in the cells treated with LiCl in a dose-dependent manner (Fig. 2C). These results indicated that the reovirus infection was inhibited by LiCl.

2.3. Antiviral effect of LiCl occurred at replication step

The antiviral effect of LiCl may occur during viral attachment, entry, or replication. Thus, we next determined which step during reovirus life cycle was affected by LiCl. In the viral attachment step, the mean viral titers ($TCID_{50}$/ml) and the mean relative viral mRNA of 0, 10, 20, 40 and 60 mM LiCl-treated cells were 3.07, 2.91, 3.10, 2.96 and 2.75, respectively (Fig. 3A), and 100.00, 98.67, 98.00, 92.33, and 91.00, respectively (Fig. 3B). In the viral entry step, the mean viral titers ($TCID_{50}$/ml) and the mean relative viral mRNA of 0, 10, 20, 40 and 60 mM LiCl-treated cells were 3.99, 3.86, 3.70, 3.58 and 3.49, respectively (Fig. 3C), 100.00 and 96.33, 97.00, 94.00, and 88.67, respectively (Fig. 3D). There were no significant differences in the mean viral titers and relative viral mRNA between control groups and treatment groups in viral attachment step and viral entry step. In the viral replication step, the mean viral titers ($TCID_{50}$/ml) of 0, 10, 20, 40 and 60 mM LiCl-treated cells were 4.17, 4.23, 3.42, 2.17 and 1.47, respectively (Fig. 3E), with the mean relative viral mRNA being 100.00, 91.00, 85.00, 35.67, and 29.67, respectively (Fig. 3F). Compare with control groups, the viral titers and relative mRNA level significantly decreased in the treatment groups in a dose-dependent manner. These results indicated that LiCl did not affect reovirus attachment and entry, but rather inhibited reovirus replication in the Vero cells.

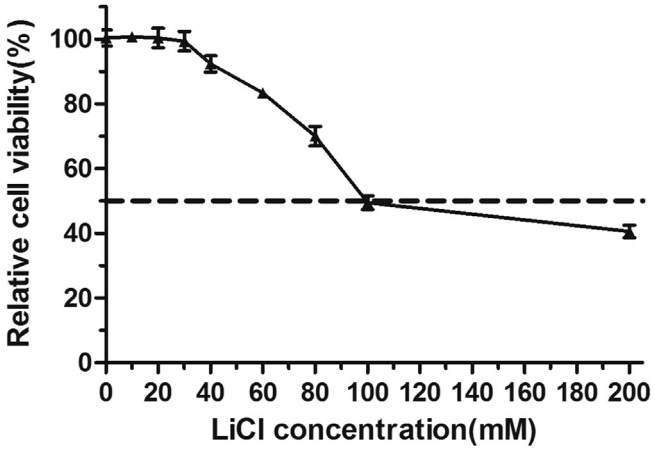

Fig. 1. The cytotoxic effect of LiCl treatment on Vero cells. The cytotoxicity of LiCl was evaluated by CCK8 following the manufacturer's instructions. Cells were treated with a series of concentrations (10, 20, 40, 50, 60, 80, 100, 150, and 200 mM) of LiCl for 24 h. The relative cell viability was calculated as (mean OD450 drug)/(mean OD450 control) × 100%.

2.4. LiCl inhibited reovirus at the early stages of replication

After confirmation of the anti-reovirus activity of LiCl, we went on to determine during which stage of replication reovirus was inhibited by LiCl. The mean viral titers ($TCID_{50}$/ml) at 0, 2, 4, 6, 8, 10, 12, 14, 16 and 18 h post-infection (hpi) from 60 mM LiCl-treated cells were 2.29, 1.83, 2.30, 2.75, 3.89, 4.39, 4.49, 4.21, 4.07 and 4.02, respectively (Fig. 4A), with the mean relative viral mRNA being 29.00, 33.67, 33.37, 48.33, 81.67, 96.67, 95.33, 99.00, 96.67 and 96.33 (sham treatment set as 100), respectively (Fig. 4B). These results indicated that LiCl inhibited reovirus replication at an early stage.

3. Discussion

An updated literature search shows that reovirus not only causes considerable economic loss in the swine industry of the United States and other countries, but also poses zoonotic potential to public health [11–15,19,21–24]. Besides treatment of depression, bipolar disorder, Alzheimer's disease, and fracture in rodent models [32–34], LiCl has the potential to be a broad-spectrum antiviral agent.

In our study, ~60 mM concentrations of LiCl showed neither significant toxicity to Vero cells nor significant impact on cell morphology. Firstly, LiCl treatment with the concentrations could inhibit reovirus infection in Vero cells. Secondly, we further confirm the antiviral activity of LiCl at different steps during viral life cycle. As shown in the results, LiCl had no effects on reovirus attachment or entry in Vero cells, which implies that LiCl does not directly affect the interaction between the viruses and cell receptors, and does not interfere the passage of viruses into cells. However, LiCl significantly inhibited reovirus replication step in Vero cells in a dose-dependent manner. Thirdly, to further investigate what stage of reovirus replication step was affected by LiCl treatment, LiCl were added at several timepoints in the viral replication step. Reovirus replication was affected after the treatment by LiCl within 8 hpi, suggesting that the early stage of viral replication is the target for the antiviral effect of LiCl. The inhibition of early stage of viral replication indicates that LiCl might inhibit the production of viral RNA and/or viral protein.

4. Conclusions

Reovirus infection in Vero cells can be inhibited by LiCl treatment in a does-dependent manner, which targets the early stage of viral replication. LiCl could be a potential drug for reovirus infection. However, the underlying mechanism of LiCl inhibiting reovirus infection needs to be further investigated.

5. Materials and methods

5.1. Virus, cell, and reagents

The MRV used in this study was serotype 3 isolated from a diarrhea pig and preserved in our laboratory. The S1 gene of the virus was cloned by RT-PCR, and the serotype was defined by nucleotide homology analysis. Vero cells were obtained from the American Type Culture Collection (ATCC) and maintained in Dulbecco's modified Eagle medium (DMEM) (Gibco, USA) containing