



(+)-Catechin inhibition of transmissible gastroenteritis coronavirus in swine testicular cells is involved its antioxidation

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ABSTRACT

Transmissible gastroenteritis virus (TGEV) causes transmissible gastroenteritis (TGE), especially in newborn piglets, which severely threatens the worldwide pig industry. In this study, (+)-catechin was evaluated for its antiviral effect against TGEV in vitro. Viability assays revealed that (+)-catechin treatment exerted a dose-dependent rescue effect in TGEV-infected ST cells, and this result was only obtained with the post-treatment application of (+)-catechin. The viral yields in (+)-catechin-treated cultures were reduced by almost three log₁₀ units. Quantitative real-time PCR analysis of the TGEV genome revealed that TGEV RNA replication was restricted after (+)-catechin treatment. Intracellular reactive oxygen species (ROS) detection showed that (+)-catechin alleviated ROS conditions induced by TGEV infection. Our results showed that (+)-catechin exerts an inhibitory effect on TGEV proliferation in vitro and is involved its antioxidation.

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1. Introduction

Transmissible gastroenteritis virus (TGEV) can infect enteric and respiratory tissues and cause transmissible gastroenteritis (TGE), which is characterized by vomiting, severe diarrhoea and dehydration. At present, TGE continues to be considered internationally as a highly contagious disease in swine, which results in a mortality rate close to 100% in newborn piglets (Cavanagh, 1996; Sola et al., 1998). A common vaccine likely cannot induce a local immune response in the small intestine and provide solid protection to piglets (Tuboly et al., 2000). Therefore, the development of effective anti-TGEV agents for controlling TGEV infection remains an important goal.

Green tea is derived from the dried leaves of *Camellia sinensis* and is one of the most commonly consumed beverages around the world (Ning et al., 2011; Pengbo et al., 2010). Approximately 70% of green tea extracts is catechins (monomeric flavonoids) (Sang et al., 2011). (+)-Catechin is one of the catechins in green tea. Many studies have shown that the catechins of green tea possess multiple pharmacological activities (Chang et al., 2003; Friedman, 2007; Song et al., 2005). Previous antiviral studies have indicated that catechins exert inhibitory effects on different types of viruses in different ways (Friedman, 2007). Typically, epigallocatechin gallate (EGCG), a subclass of catechins, can efficiently inhibit the entry of hepatitis B virus (HBV) into immortalized

human primary hepatocytes by inducing clathrin-dependent endocytosis (Huang et al., 2014). The antiviral effect of EGCG on human immunodeficiency virus type 1 (HIV-1) has been shown for several steps in the HIV-1 life cycle, including a destructive effect on viral particles, post-adsorption entry and reverse transcription in acutely infected monocytoic cells (Yamaguchi et al., 2002). The antioxidant and anti-inflammatory activities of (+)-catechin have been previously observed in several studies (Bragança de Moraes et al., 2014). However, the impact of (+)-catechin on viral infections has not been previously investigated in detail.

The present study aimed to investigate the antiviral potential of (+)-catechin. We used a swine testicle (ST) cell line to assess the protective effects of (+)-catechin on TGEV infection in terms of viral replication and cell survival. In addition, the antiviral mechanism of (+)-catechin was preliminarily studied.

2. Materials and methods

2.1. Compounds

(+)-Catechin was purchased from Shanghai Winherb Medical Technology Co., Ltd. (China). It was dissolved in dimethyl sulfoxide (DMSO) and stored at −20 °C. MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide), 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) and dihydroethidium (DHE) were obtained from Beyotime Institute of Biotechnology (Jiangsu, China).

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2.2. Cells and virus

Swine testicle (ST) cells were grown in minimal essential medium (MEM; GIBCO, UK) with 10% foetal calf serum (HyClone, China). The TGEV H16 strain was purchased from the National Control Institute of Veterinary Bioproducts and Pharmaceuticals (Beijing, China) and grown in ST cells.

2.3. MTT assay

The cytotoxicity and antiviral effects were tested using the MTT assay. Different concentrations of compounds were added to ST cell monolayers in 96-well culture plates with or without TGEV adsorption. Untreated cells served as the control. All of the cells were maintained at 37 °C in 5% CO₂. The supernatant was carefully removed from the plate without disturbing the attached cells, and 50 µL of MTT (2 µg/mL) was added to each well. After the plate was incubated at 37 °C for 4 h, the excess MTT was removed, and 200 µL of DMSO was added to each well. The 96-well culture plate was placed in an electronic oscillator for 10 min to dissolve the formazan crystals. The light absorbance of each well was measured at 490 nm in a microplate reader (Thermo).

2.4. Assays of antiviral activity

To investigate the effect of (+)-catechin on TGEV reproduction, three different assays were performed. First, TGEV and (+)-catechin were mixed and incubated at 4 °C for 1 h, and the mixture was then added to ST cell monolayers in culture plates at 37 °C in 5% CO₂ for 1 h. After 1 h of adsorption, the unadsorbed virus and compounds were replaced with fresh medium. Second, (+)-catechin was added to ST cells 4 h before TGEV adsorption. The remaining steps were the same as those used in the first assay. Third, TGEV was adsorbed for 1 h at 37 °C in 5% CO₂. The unadsorbed viruses were removed, and media containing different concentrations of (+)-catechin were added. Mock-treated cells and cells treated with only TGEV served as controls in all of the assays. The cell morphology was observed, and the antiviral effect was analysed after 48 h using the MTT assay. All of the tests were performed in triplicate.

2.5. Virus titration

The culture supernatants were collected for virus titration. The supernatants were serially diluted 10-fold from 10^{-1.0} to 10^{-11.0} and added to ST cell monolayers in 96-well culture plates. Each dilution was added to eight wells. The TCID₅₀ was calculated by the Karber method after 48 h of infection.

2.6. RNA extraction and quantitative real-time PCR

ST cells were cultured in six-well culture plates. TGEV was added to the ST cells at 70–80% confluence in the plates, with the exception of the negative control. After 1 h of incubation at 37 °C in 5% CO₂, the medium was removed, and fresh medium containing (+)-catechin was added. Fresh medium with the same concentration of EDTA was added to the positive-control and negative-control wells. TGEV-infected cells were collected 40 h after viral infection to evaluate the inhibitory effects of (+)-catechin on TGEV replication. RNA extraction, cDNA synthesis and quantitative real-time PCR were performed according to a previous report (He et al., 2012b). The Ct method was employed to analyse the data, and the amount of RNA in the samples was normalized to that of β-actin.

2.7. Detection of intracellular reactive oxygen species (ROS)

The intracellular ROS was measured using an oxidation-sensitive fluorescent probe (DCFH-DA). After TGEV infection and (+)-catechin

treatment for 24 h, ST cells were washed with PBS and incubated with 10 µM DCFH-DA for 20 min at 37 °C. H₂O₂ served as a control. The extracellular DCFH-DA was washed three times with PBS. The fluorescence was observed and recorded using a fluorescence microscope (Nikon, Japan).

The intracellular superoxide anion was measured using a fluorescent dye DHE. TGEV infection and (+)-catechin treatment were the same as those used in the DCFH-DA assay. ST cells were washed with PBS and incubated with 2.5 µM DHE for 30 min at 37 °C. The fluorescence was measured using a luminescence plate reader (Perkin Elmer, America). The light absorbance of each well was measured at 610 nm.

3. Results

3.1. Measurement of cytotoxic (+)-catechin concentrations

Before (+)-catechin could be used for antiviral studies, a non-toxic dose was determined by adding different concentrations to ST cells. (+)-Catechin was serially diluted two-fold from 1280 µM to 10 µM. At (+)-catechin concentrations less than 320 µM, there was no difference in the cellular morphology and density between the (+)-catechin-treated and control cells. ST cells treated with 640 µM (+)-catechin presented evident morphological changes, including cell shrinkage, cell size reduction, turning round and shedding.

The (+)-catechin cytotoxic concentration was determined by the MTT assay. Consistent with the morphological observations, no significant difference was obtained at (+)-catechin concentrations less than 320 µM. However, the cell viability observed with 640 µM (+)-catechin was decreased compared with that of the cells treated with (+)-catechin at concentrations less than 320 µM (Fig. 1).

According to the test results, we ascertained that a dose of (+)-catechin less than 320 µM was safe.

3.2. Antiviral effect of (+)-catechin

After the cytotoxicity test, we tested the antiviral effect of (+)-catechin. To determine which steps in the viral life cycle were affected by (+)-catechin, the antiviral effect was tested through three different approaches. (+)-Catechin was added before, during and after the

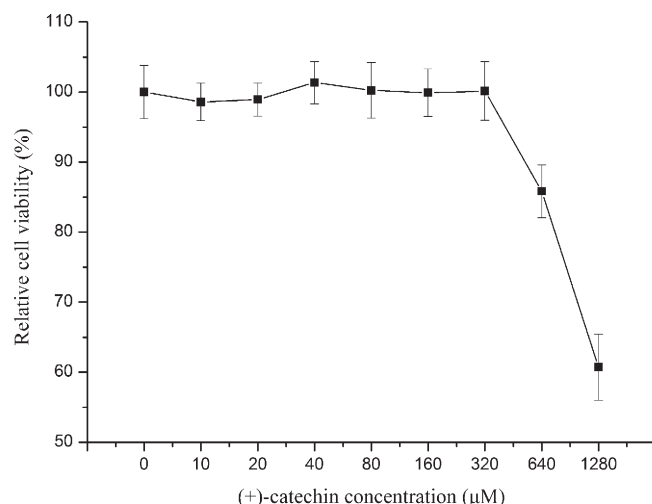


Fig. 1. (+)-Catechin cytotoxicity. Different concentrations of (+)-catechin were added to ST cell monolayers in 96-well plates. The tested concentrations were between 20 and 1280 µM. The MTT assay was used to test the cell viability after 48 h of incubation at 37 °C. The relative cell viability was calculated by comparing the O.D.₄₉₀ values of the (+)-catechin-treated cells with those of the non-treated control cells (set to 100%). The relative cell viability obtained after incubation with different concentrations of (+)-catechin is provided. The cell viability of the cells treated with (+)-catechin at concentrations less than 320 µM was not different from that of the non-treated control cells. The results represent the means ± SD.

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