Antiviral Research 130 (2016) 93–100

Contents lists available at ScienceDirect

Antiviral Research

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

Antiviral effect of resveratrol in ducklings infected with virulent duck enteritis virus

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ARTICLE INFO

Article history:
Received 10 December 2015
Received in revised form
16 March 2016
Accepted 29 March 2016
Available online 31 March 2016

Keywords:
Resveratrol
Antiviral activity
Duck enteritis virus

ABSTRACT

Duck enteritis virus (DEV) is a double-stranded DNA virus belonging to the alphaherpesvirinae subfamily of the *herpesviridae*. Although vaccines were wildly used in controlling this disease, some infection could still not be prevented and led to significant economic losses as a result of mortality and decreased egg production. However, there is no antiviral drug against DEV. Resveratrol was identified to exert its antiviral activity by inhibiting the DEV replication in preliminary investigations. In the present study, we confirmed that resveratrol significantly reduced the mortality of ducklings which infected with a virulent strain of DEV. With resveratrol treatment, the survival rate increased by almost 80% at 8 days post infection (dpi). Pathological symptoms of ducklings caused by DEV were also relieved by resveratrol. The virus load in blood and tissues were effectively depressed when compared with the untreated group. In the assay of immune cytokines, the resveratrol exerted a dual-regulation effect. These results suggest that resveratrol is expected to be a new alternative control measure for DEV infection.

1. Introduction

Duck enteritis virus (DEV), also called duck plague virus, is a double-stranded DNA virus belonging to the alphaherpesvirinae subfamily of the *herpesviridae* (Kaleta, 1990). DEV was emerged from 1923 in Holland, and then spread to many countries (Islam et al., 2005). This disease occurs in all members of the family *Anatidae* of the *order Anseriformes* and transmitted by direct or indirect contact in susceptible flocks, leading to significant economic losses as a result of mortality and decreased egg production (Gast, 2003). Vaccines were wildly used in controlling this disease, while some infection could still not be prevented (Mondal et al., 2010; Wang and Osterrieder, 2011; Wang et al., 2011). However, there is no antiviral drug against DEV. Thus, there is a need to develop effective antiviral agents to treat DEV infection.

Resveratrol, a non-flavonoid polyphenol compound wildly existing in several higher plants, has been reported to have a wide range of bioactivities against many diseases, such as cancers, myocardial infarction, inflammation, immunity, stroke, brain damage, diabetes and viral diseases (Baur J A, Sinclair D A, 2006). A variety of studies have reported that resveratrol inhibited the replication of HSV-1 and HSV-2 (Docherty et al., 2004). Furthermore, the antiviral activity of resveratrol has also been reported in the studies of varicella zoster virus (Docherty et al., 2006), human cytomegalovirus (Eversion et al., 2004), Epstein-Barr virus (De Leo et al., 2012), human immunodeficiency virus (Zhang et al., 2009), influenza A virus (Palamara et al., 2005) and African swine fever virus (Galindo et al., 2011).

The anti-DEV activity of resveratrol was evaluated for the first time in our laboratory and the preliminary results showed that it could effectively inhibit the virus replication in vitro (Xu et al., 2013). In this study, we studied the antiviral activity of resveratrol in ducklings infected with virulent DEV in order to develop a new alternative control measure for DEV infection.

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http://dx.doi.org/10.1016/j.antiviral.2016.03.014
0166-3542/

2. Materials and methods

2.1. Compounds

Resveratrol, bought from Sigma with a purity of 98%, was dissolved in 0.5% carboxymethylcellulose-phosphate buffered saline (SCMC-PBS) before use.

2.2. Virus and ducklings

The DEV CH virulent strain (DEV-CHv) was provided by Prof. Anchun Chen of the Institute of Preventive Veterinary Medicine, Sichuan Agricultural University (Chengdu, P.R. China). Viruses were propagated in 11-day-old duck embryo eggs and the 50% egg lethal dose (ELD_{50}) was measured as $10^{-9.16}$/mL. Newly hatched ducklings were purchased from remote hatchery (No special antibody against DEV) of Ya'an, China. Ducklings were maintained under normal daylight, feeding with a standard commercial diet and water ad libitum.

2.3. Ethics statement

All procedures involving animals and their care in this study were approved by the Ethics Committee of Sichuan Agricultural University according to The Regulation of Experimental Animal Management (State Scientific and Technological Commission of the People's Republic of China, No. 2, 1988) and The Interim Measures of Sichuan Province Experimental Animal Management (Science and Technology Bureau of Sichuan, China, No.25,2013).

2.4. Experimental design

Sixty-five 21-days-old ducklings were randomly divided into five groups. Each duck was subcutaneously injected with 0.1 mL DEV suspension at a dose of 1×10^7 ELD_{50}, except the normal control group. The infected ducklings were immediately received the resveratrol solutions orally at a dose of 200 (RV-H), 100 (RV-M) and 50 mg/kg body weight (RV-L) daily for 7 days, respectively. The untreated and normal groups were given the same volume of SCMC-PBS. All the test groups were maintained for 2 weeks. The pathological symptoms and mortality were monitored throughout the study period. The rearing conditions were based on the Guidelines of the International Committee on Laboratory Animals.

2.5. Analysis of the viral load by real-time PCR

Real-time fluorescent quantitative PCR (FQ-PCR) was used to detect the viral load in blood. The total DNA samples were isolated from venous blood of DEV-infected ducklings which were treated with or without resveratrol by Guanidine Hydrochloride method (Fang et al., 2002). The FQ-PCR was analyzed by using Premix Ex TaqTM (Probe qPCR) (TaKaRa) with a Bio-Rad CFX96™ Manager software system according the method described in the reference (Xu et al., 2013).

2.6. Pathological analysis

Pathological lesions of DEV-infected ducklings treated with or without resveratrol were observed. The hemorrhagic injuries of digestive system including esophagus, duodenum and small intestine were recorded. Liver, kidney, lung, spleen and bursa of fabricius were collected, fixed by 4% paraformaldehyde. Hematoxylin-eosin (HE) staining was performed according to standard procedures. The sections were stained with HE (NJJCTECH, D006) and observed under the digital microscope (Nikon eclipse 80*i*). Three slides from different part of each tissue (3 ducklings per group) were analyzed. The whole lesions for each tissue were scored by multiplying the degree of severity (0 = no lesions, 1 = mild lesions, 2 = moderate lesions, and 3 = severe lesions) with the extent of lesions (1 = low extent, 2 = intermediate extent, and 3 = large extent) (Song X et al., 2015). Liver and spleen were assayed by transmission electron microscopy (TEM) in the Analytical & Testing Center Sichuan University, P. R. China.

2.7. Serum cytokines assay

Serum samples (three ducklings per group) separated from the venous blood of DEV-infected ducklings which were treated with or without resveratrol. Then, the concentrations of cytokines including IFN-α, IFN-γ, IL-2, IL-4 and IL-12 in serum were detected by using duck ELISA kit according to the manufacturer's instructions (ShangHai Lengton Bioscience Co.,LTD). Briefly, serial 2-fold dilutions of standard cytokine were prepared with standard diluent, and then 50 μL of each dilution was added into the antibody-coated microtiter plate. The 40 μL of each serum sample together with 10 μL biotinylated antibody were added. The plate sealed by a closure membrane was allowed to incubate at 37 °C for 30 min, followed by washing five times with wash buffer. HRP-conjugate reagent was then added into each well and the plate was incubated at 37 °C for 30 min. After washing, 50 μL chromogen solution A and B were added and the plate was incubated at 37 °C for 15 min. Finally, 50 μL stop solution was added and the optical density at 450 nm was measured by a microplate reader (Bio-Rad, USA). The sample concentration was calculated according to the constructed standard curve.

3. Results

3.1. Resveratrol reduced mortality of ducklings infected with DEV

The survival rate of ducklings in each group was shown in Table 1. There were no deaths in each group at 4 days post-infection (dpi), but at 5 dpi the ducklings began to die in the untreated group, at the same time there were no deaths in the resveratrol-treated groups. The resveratrol-treated groups exhibited high protection rate (80%) in DEV-infected ducklings at 8 dpi, while the ducklings in the untreated group were died out. The survival rates were decreasing in resveratrol-treated groups after 7 dpi (stopped to give resveratrol). Surprisingly, there were 20%, 40%, 50% ducklings still

Table 1
Resveratrol treatment reduced the mortality of DEV-infected ducklings.

Days post infection	Survival rate (%)				
	RV-H	RV-M	RV-L	Untreatment	Normal
1	100	100	100	100	100
2	100	100	100	100	100
3	100	100	100	100	100
4	100	100	100	100	100
5	100	100	100	70	100
6	80	100	100	60	100
7	80	90	80	40	100
8	80	80	80	0	100
9	80	60	80	0	100
10	60	60	70	0	100
11	50	60	60	0	100
12	40	50	60	0	100
13	40	40	50	0	100
14	20	40	50	0	100

Survival rates of the DEV-infected ducklings treated with resveratrol (RV-H, RV-M, RV-L, respectively) and untreated were recorded at 14 dpi (n = 10, in each group).