Food Sci. Biotechnol. 25(S): 103-106 (2016)
DOI 10.1007/s10068-016-0105-8

Food Science
and
Biotechnology

Protective Effect of Resveratrol against Pseudorabies Virus-induced Reproductive Failure in a Mouse Model

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Received November 21, 2015
Revised February 27, 2016
Accepted February 29, 2016
Published online March 31, 2016

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pISSN 1226-7708
eISSN 2092-6456

Abstract Resveratrol (RES), a natural polyphenol that is abundant in grapes, exerts anti-inflammatory, anti-oxidative, and antiviral bioactive effects. Protective effects of RES against pseudorabies virus (PrV)-induced reproductive failure were investigated in a mice model. Injection of PrV partially induced stillbirth and abortion, and caused poor growth of progeny. Treatment with RES attenuated the reproductive failure induced by the virus with recovery of the serum progesterone level. RES improved the growth performance of newborn mice. RES can attenuate the reproductive failure induced by PrV in mice.

Keywords: resveratrol, antivirus, pseudorabies, reproductive failure

Introduction

Resveratrol (RES), a naturally occurring plant antibiotic known as phytoalexin, is found in plants, nuts, and fruits, especially grapes (1). Numerous studies have reported that RES exerts protective effects against organ injury and dysfunction due to anti-inflammatory and antioxidant properties (2-6). In recent years, RES has also been considered as a potent polyphenolic compound against human and animal viruses (7-9). Thus, RES probably has an ability to inhibit the activity of viruses and to reduce the risk of infection in animals.

The pseudorabies virus (PrV) is a contagious herpes virus that can infect mammals, including ruminants, carnivores, rodents, and pigs (10,11). The disease caused by PrV is one of the most economically important viral diseases in the swine industry, and PrV infection causes reproductive failure, such as abortion, stillbirth, mummies, and infertility in sows. There is no specific treatment for acute PrV infection. Vaccination and concurrent antibiotic therapies are recommended for alleviation of clinical signs in pigs (12,13). Therefore, development of effective antiviral agents for prevention of PrV infection is necessary. Based on antiviral properties of polyphenols and resveratrol (9,14), protective effects of RES against reproductive failure induced by PrV in mice were studied.

Materials and Methods

Virus and reagents PrV strain Bartha K-61, obtained from the College of Veterinary Medicine, Northeast Agricultural University, Harbin, Heilongjiang, China was propagated in porcine kidney epithelial (PK15) cells. Resveratrol (≥99%) and carboxymethyl-cellulose (CMC) sodium were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animals All animal protocols were performed in accordance with guidelines of the Animal Care and Use Committee of Hunan Agricultural University. One hundred 7-week-old female ICR mice weighing, on average, 27.3±0.9 g were purchased from Hunan Silaikejingda Laboratory Animal Co., Ltd. (Changsha, Hunan, China) in November of 2014. Mice were housed at a constant temperature of 24°C with 35-45% humidity under a 12 h light/dark cycle and free access to food and water.

Treatment with resveratrol and challenge with PrV After 5 days of acclimatization, mice were randomly assigned to 5 groups (n=20) designated as CTL, PrV, RES50, PrV plus RES25, and PrV plus RES50. Mice in the CTL and PrV groups were administered a 0.5%

carboxylmethylcellulose (CMC) solution at 100 μL per mouse once a day via gavage. Mice in the PrV plus RES25, PrV plus RES50, and RES50 groups received RES at 25, 50, and 50 mg/kg of body weight (BW) suspended in a 0.5% CMC solution. The RES dosage was determined based on results of a pilot trial. Mice BW were recorded every 3 days. After 16 days, all female experimental mice were caged with adult male ICR mice at a 2:1 ratio for 4 days to achieve pregnancy. The vaginal plug was checked every morning and the day with the presence of a plug was defined as day 1 of pregnancy. On day 7 of pregnancy, mice from PrV, PrV plus RES25, and PrV plus RES50 groups were intraperitoneally inoculated with 100 μL of PrV (100 $TCID_{50}$, dissolved in Dulbecco's Modified Eagle Medium (DMEM; HyClone Laboratories, South Logan, UT, USA)). CTL and RES50 group mice were injected with 100 μL of DMEM. The PrV injection dosage that did not cause death of pregnant mice was determined based on a preliminary trial.

Detection of PrV nucleotides using PCR The brain, liver, placenta, and ovaries were collected for detection of PrV nucleotides. DNA was extracted from tissues using a TaKaRa MiniBEST Universal Genomic DNA Extraction Kit (Takara Bio Inc., Shiga, Japan) following manufacturer instructions. Purified DNA was amplified using a PCR Amplification Kit (Takara Bio Inc.,) and primers specific for the PrV glycoprotein E (gE) gene (Forward: 5'-CTTCCACTCGCAGCTCTTCT-3'; Reverse: 5'-TGGTAGATGCAGGGCTCGTA-3')(15). Reaction conditions were 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 60°C for 15 s, and 72°C for 20 s. PCR products were analyzed using 1.2% agarose gel electrophoresis with a Mini-PROTEAN Tetra cell (Bio-Rad Laboratories, Hercules, CA, USA).

Assessment of reproductive performance On day 14 of pregnancy, blood samples were collected from 10 mice per group in anticoagulant-free tubes and allowed to stand for 30 min at room temperature for coagulation. Serum was obtained via centrifugation at 1,500x*g* for 10 min by using a Sorvall Stratos Centrifuge (Thermo Fisher, Waltham, MA, USA), and stored at −80°C until use (16). The serum progesterone level was measured using a progesterone radioimmunoassay kit (Cisbio Bioassays, Codolet, France) following manufacturer instructions. Mice were dissected, followed by blood collection, and uteri were opened for recording of numbers of live, dead, absorbed fetuses, and the rate of abortion (number of aborted mice/total number of mice×100%). Other mice were kept until delivery and litter size, including number of live pups, and average daily gain (ADG) of new born mice were recorded.

Statistical analysis Results were expressed as means±standard deviation (SD). Significant differences were determined using a one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test in SPSS 19.0 (IBM Corp., Armonk, NY, USA) at $p<0.05$ for significance.

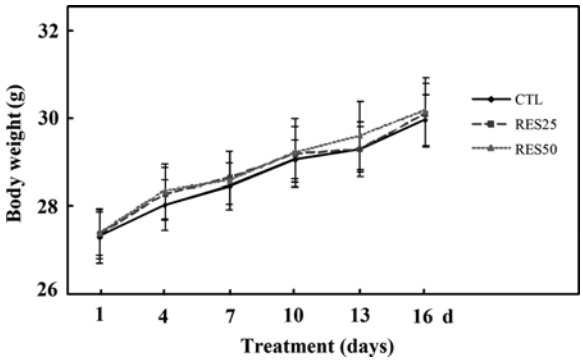

Fig. 1. Effect of RES on growth of female mice. Mice were treated with RES at 25 and 50 mg/kg BW/d for 16 days and BW values were recorded every 3 days. Values represent a mean±SD (n=20). No significant ($p<0.05$) differences were observed between different groups. CTL, control; RES, resveratrol.

Results and Discussion

RES attenuated PrV-induced reproductive failure in mice Protective effects of resveratrol supplementation during pregnancy for reproductive performance of mice challenged with PrV, a virus that causes severe reproductive failure, were examined. No natural animal death occurred during experimentation. The effect of RES on growth performance of mice was investigated. No significant ($p<0.05$) differences in ADG values were observed between control group mice and mice in groups treated with 25 and 50 mg/kg BW (Fig. 1).

The PrV virus can cross the uterus and placenta boundaries and infect the fetus, causing abortion, stillbirth, or birth of weak litters. Mice were challenged with PrV on day 7 of gestation and reproductive outcomes were recorded on day 14 of pregnancy. PrV infection caused a high abortion rate of 40% in mice, and fetal survival was improved with resveratrol supplementation (Fig. 2A). Abortion rates were reduced to 30 and 15% for mice in groups treated with RES at 25 and 50 mg/kg BW, respectively. No abortion was observed in control group mice and in mice in the group treated with RES only at 50 mg/kg BW. Moreover, PrV infection also caused a decrease in litter size to 10.5±1.6, the average number of live pups upon delivery. With RES supplementation, mice challenged with PrV delivered a litter size of 12.4±2.2 for mice in the RES25 plus PrV group, and 13.8±1.6 for mice in the RES50 plus PrV group (Fig. 2B). Feeding rats suffering hypoxia with resveratrol improved fetal outcomes, and resveratrol was detected in the plasma of hypoxic rats and fetuses (17). Therefore, fetal loss induced by PrV infection can be rescued using RES, which can cross the placental boundary and act against viral infection of the placenta and fetus directly.

In this study, a viral pathogen was detected in the brain, liver, placenta, and ovaries of mice infected with PrV using a PCR assay. Only a weak viral gE gene signal was detected in brain tissue.

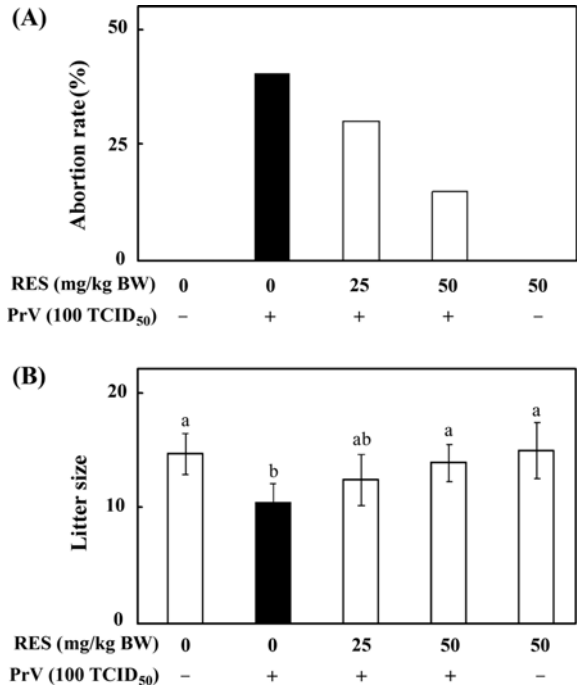

Fig. 2. RES attenuated PrV-induced reproductive failure in mice. (A) RES-treated mice were challenged with PrV on day 7 of pregnancy and the abortion rate (number of aborted mice/total number of mice×100%) was recorded on day 14 of gestation for 10 mice in each group. (B) RES-treated mice were challenged with PrV on day 7 of pregnancy and the litter size was recorded upon delivery for 10 mice in each group. Data indicate values (mean±SD) for the group treated with PrV alone (black bar) and other groups (white bar). Means with different small letters differ significantly (p<0.05). RES, resveratrol; PrV, pseudorabies virus.

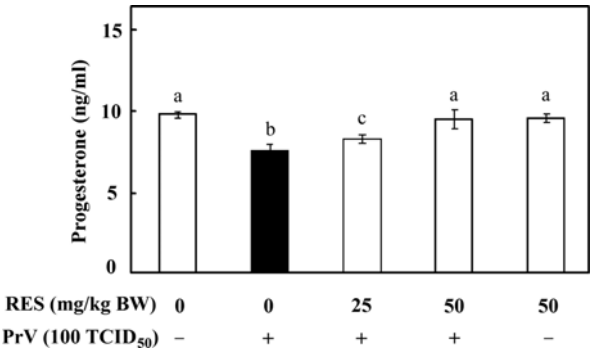

Fig. 3. RES increased serum progesterone levels in PrV-infected mice. RES-treated mice were challenged with PrV on day 7 of pregnancy and the serum progesterone level was determined 7 days post-PrV challenge. Data are presented as means±SD (n=10) for the group treated with PrV alone (black bar) and other groups (white bar). Means with different small letters differ significantly (p<0.05). RES, resveratrol; PrV, pseudorabies virus.

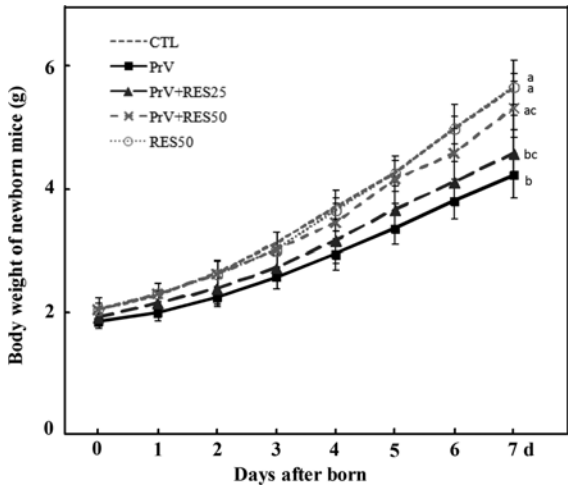

Fig. 4. RES increased ADG values of newborn mice. Body weights of new born mice were determined each day in the first week after birth. Values represent a mean±SD, and values with different small letters indicate a significant difference (p<0.05). CTL, control; PrV, pseudorabies virus; RES, resveratrol.

RES increased serum progesterone levels in PrV-infected mice The progesterone status is critical to pregnancy outcomes in mammals (18) and progesterone is considered to be an important endogenous steroid for maintenance of pregnancy and prevention of abortion (19). Low serum progesterone levels are associated with fetal resorption in an abortion-prone mice model (20). Resveratrol has been reported to enhance progesterone secretion and expression of luteinization-related genes in the ovaries in a rat model (21). Thus, the level of progesterone in mice sera was determined. PrV infection significantly (p<0.05) decreased the level of progesterone in mouse sera, compared with controls, (Fig. 3). However, the serum progesterone level in PrV-infected mice was recovered with RES supplementation in a dose-dependent manner, and attained a normal control level in mice administrated RES at 50 mg/kg BW daily. Mice treated with RES at 50 mg/kg BW alone showed no significant (p>0.05) difference, compared with control mice. Thus, RES attenuated the fatal loss induced by PrV in mice, partially via maintenance of the progesterone level in maternal sera.

Prostaglandins (PGs) and analogues are widely used for both delivery and abortion via stimulation of uterine smooth muscle contraction. Euterine exposure to RES can inhibit the uterine smooth muscle contraction induced by $PGF_{2\alpha}$ both *in vitro* and *in vivo* (22). Several viruses are known to stimulate cyclooxygenase (COX)-2 mediated PGE_2 production (23-25). The effect of PrV on production of PGs, and the role of RES in uterine smooth muscle contraction in the animal model used in this study need further investigation.

RES increased growth performance in newborn mice Transplacental and perinatal infection occurs in sows infected with PrV, resulting in poor outcomes for newborn piglets (26). For study of the effects of RES on the progeny of mice, ADG values of newborn mice were recorded in the first postnatal week. ADG values for baby mice from dams with PrV infection were significantly (p<0.05) lower than for control group mice. However, RES supplementation attenuated poor growth performance and improved ADG values of neonates

delivered by PrV infected mice in a dose-dependent manner (Fig. 4). There was no significance (p>0.05) between control group mice and mice in the group treated with 50 mg/kg BW of RES alone. Thus, RES showed beneficial effects against PrV-induced reproductive failure in pregnant mice, and improved the growth performance of newborn mice.

Acknowledgments This work was partly supported by grants from the National Natural Science Foundation of China (31272630, 31371683, and 31302005). The laboratory staff of the College of Animal Science and Technology, Hunan Agricultural University, provided valuable assistance.

Disclosure The authors declare no conflict of interest.

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