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Original article

Resveratrol treatment inhibits acute pharyngitis in the mice model through inhibition of PGE2/COX-2 expression

Zhaoyan Yu, Wei Xu, Haibo Wang *

Department of Otolaryngology-Head and Neck Surgery, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, Shandong 250021, China

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ABSTRACT

The aim of the present study was to investigate the effect of resveratrol on acute pharyngitis in the mice models induced by xylene and carrageenan treatment. The mice treated with various doses of resveratrol (5, 10, 15, 20 and 30 mg/kg) showed inhibition of edema in a dose dependent manner. The edema formation was reduced by 67% in the mice treated with 20 mg/kg of resveratrol compared to those in the control group. A significant ($P < 0.02$) reduction of paw swelling was observed in the mice treated with 20 mg/kg dose of resveratrol compared to the control group. The inhibition of paw swelling in mice was also caused by votalin by the extent of reduction was significantly ($P < 0.02$) lower compared to the resveratrol treatment. In the mice model of paw swelling, treatment with 20 mg/kg doses of resveratrol significantly ($P < 0.02$) reduced the expression of PGE2 compared to the control group. On the other hand, resveratrol played a vital role in the inhibition of carrageenan induced increase in the expression of COX-2 in mice. The inhibition in the COX-2 expression by 20 mg/kg doses of resveratrol was significantly higher compared to the known drug, votalin. Thus the current study revealed that resveratrol treatment inhibits acute pharyngitis in the mice model through inhibition of PGE2/COX-2 expression. Thus resveratrol can be used for the treatment of acute pharyngitis.

1. Introduction

Acute pharyngitis which is characterized by severe inflammation in the mucous lining of the pharynx is caused by viral and bacterial infections in the upper respiratory tract. However, the viral infections are found to be more predominant compared to the bacterial infections (Shah et al., 2011). The symptoms of acute pharyngitis include fever, pain at the time of swallowing and sore throat (Bisno, 2011). Bacterial super-infections are responsible for the worsening of acute pharyngitis following initial stage of mild infection. Previous treatments of acute pharyngitis with fever, pain or inflammation include non-steroidal anti-inflammatory drugs and steroids (Tasar et al., 2008; Schams and Goldman, 2012), which have potential risks and side-effects (Mullarkey, 2011). The commonly used treatment for pharyngitis involves administration of steroidal and non-steroidal drugs with anti-inflammatory properties. However, these drugs have the harmful side effects and influence human health (Huang et al., 2012). Over the decades, traditional Chinese medicine has been used for the treatment of acute pharyngitis (Sun et al., 2011). The herbs with the anti-inflammation, anti-viral and anti-pyritic properties were used commonly for the treatment of acute pharyngitis. Thus natural products particularly those with anti-oxidant properties have a scope for the treatment of pharyngitis (Antonisamy et al., 2015; Balamurugan, 2015; Rathi et al., 2015; Nandhini and Stella Bai, 2015; Kalaiselvi et al., 2016; Neelamkavil and Thoppil, 2016). Resveratrol one of the constituents present ingrapes and wine is produced when the plants are under stress (Frémont, 2000). Studies have revealed a correlation between resveratrol presence and cardiovascular diseases (Ciolino et al., 1998). Resveratrol plays an important role in the prevention of heart diseases, inhibition of carcinoma and suppression of inflammatory disease (Martinez and Moreno, 2000; Yang et al., 2016; Vasamsetti et al., 2016; Mathieu et al., 2016). There are reports that resveratrol treatment inhibits the lipoprotein oxidation and accumulation of platelets. Resveratrol treatment also inhibits activity of various enzymes

* Corresponding author at: Department of Otolaryngology-Head and Neck Surgery, Shandong Provincial Hospital Affiliated to Shandong University, Jingwuweiqi road, Jinan, Shandong 250021, China.
E-mail address: haibowang12@hotmail.com (H. Wang).

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associated with the process of DNA replication (Fontecave et al., 2000; Fu et al., 2017; Robb et al., 2017). In the present study effect of resveratrol on the acute pharyngitis in mice model prepared by administration of xylene and carrageenan was investigated. Resveratrol significantly inhibited the acute pharyngitis in the mice model.

2. Materials and methods

2.1. Chemicals and reagents

Votalin and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The assay kits for cycloxygenase-2 and prostaglandin E2 were purchased from (NEN Life Science Products, Inc., Boston, MA, USA). Xylene and carrageenan were supplied by (Santa Cruz Biotechnology, CA).

2.2. Animals

Nu/Nu female mice (4–6 weeks old) were purchased from the Charles River Laboratories (Wilmington, MA, USA). All the animals were adopted to laboratory environment before 1 week of the start of actual experiment in a sterile and pathogen-free environment with food and water ad *libitum*. All the experimental procedures involving animals were performed in accordance with State and Federal laws, standards of the US Department of Health and Human Services, and guidelines established by Tulane University Animal Care and Use Committee.

2.3. Preparation of mice ear edema model

Thirty five mice were assigned randomly to seven groups of 5 animals each; Normal, model and five treatment groups treated with 5, 10, 15, 20 and 30 mg/kg doses of resveratrol. The mice in the model and five treatment groups were injected with 50 mg/kg of votalin in ear. The mice in the normal control group were injected with equal volume of normal saline. The paw edema mice model was prepared by injecting Carrageenan (0.1 ml, 1%w/v) into the hind paw of the mice.

2.4. Hematoxylin and eosin (H&E) staining

The animals were anesthetized using chloral hydrate and then sacrificed after completion of treatment. For each of the animal tissue sections were extracted from the paw of right leg. The extracted tissue sections were put into formalin solution and kept there for 30 h. Subsequently the tissue sections were subjected to paraffin embedding cut into thin sections. The paraffin embedded sections were deparaffined in boiling xylene and subsequently subjected to H&E staining. The stained tissue sections were examined using microscope (magnification, x400; Olympus BX41, Tokyo, Japan) to observe the alterations in tissues.

2.5. Analysis of prostaglandin E2 and cyclooxygenase-2

The extracted tissue sections from the paw of right leg of mice were subjected to homogenization using ice-cold normal saline. The ELISA kit was used for the analysis of the level of prostaglandin E2 and cyclooxygenase-2 in the tissue homogenates.

2.6. Statistical analysis

All the data presented are the mean of ±SD. Analysis of the data was performed using the unpaired Student's *t*-test (Graph Pad Prism V.4). The *p*-values of <0.05 were considered statistically significant.

3. Results

3.1. Resveratrol treatment inhibits the edema of ear induced by xylene in mice

The mice treated with various doses of resveratrol (5, 10, 15, 20 and 30 mg/kg) showed inhibition of edema in the ear caused by xylene in a dose dependent manner. The edema inhibition was found to be significant at a dosage of 20 mg/kg of resveratrol. The edema formation was reduced by 67% in the mice treated with 20 mg/kg of resveratrol compared to those in the control group. On the other hand, treatment with votalin could only reduce the edema formation by 38% (Fig. 1).

3.2. Resveratrol treatment inhibits the swelling of paw in thexylene induced mice model

The carrageenan induced paw swelled mice were treated with 5, 10, 15, 20 and 30 mg/kg concentrations of resveratrol. A significant ($P < 0.02$) reduction of paw swelling was observed in the mice treated with 20 mg/kg dose of resveratrol compared to the control group. The inhibition of paw swelling in mice was also caused by

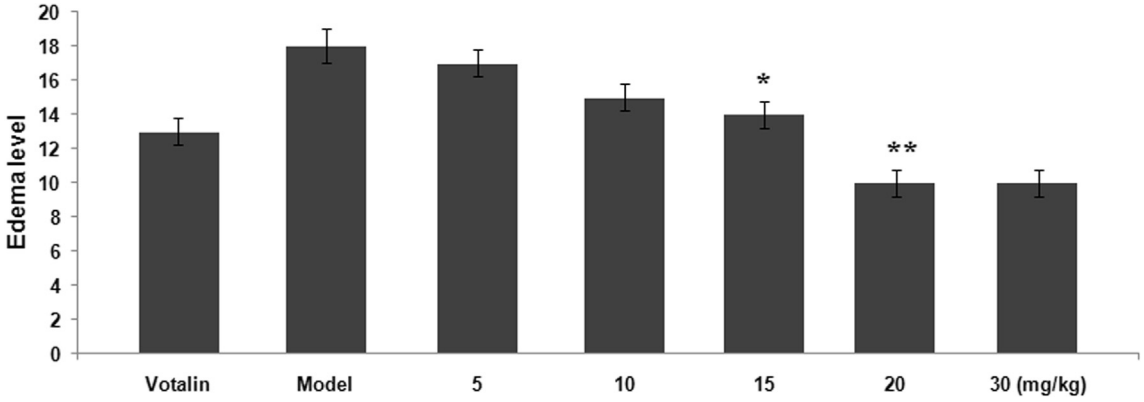

Fig. 1. Resveratrol inhibits swelling of ear in the mice administered with xylene. The mice administered xylene were treated with 5, 10, 15, 20 and 30 mg/kg doses of resveratrol and then edema formation was analyzed. The data presented are the mean ± SD.

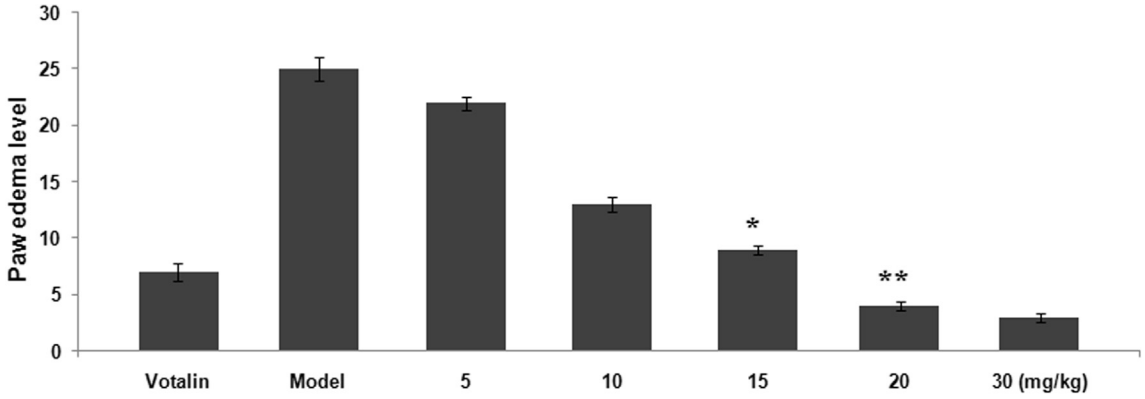

Fig. 2. Resveratrol inhibits swelling of paw in the mice administered with carrageenan. The mice administered carrageenan were treated with 5, 10, 15, 20 and 30 mg/kg doses of resveratrol and then edema formation in the paw was analyzed. The data presented are the mean ± SD.

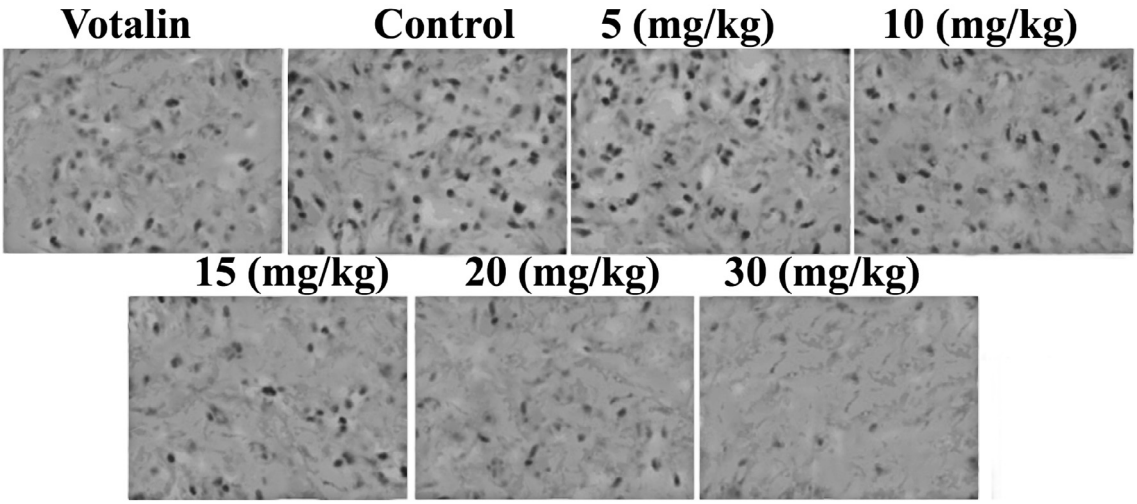

Fig. 3. Resveratrol inhibits neutrophil aggregation in the paw of mice administered with carrageenan. The mice were administered with carrageenan followed by treatment with 5, 10, 15, 20 and 30 mg/kg doses of resveratrol. The paw tissues were extracted and then examined for presence of neutrophil aggregation.

votalin by the extent of reduction was significantly ($P < 0.02$) lower compared to the resveratrol treatment (Fig. 2).

3.3. Resveratrol treatment inhibits the population of inflammatory cells present in therat paws

Examination of the tissue samples from mice paw administered with carrageenan revealed a significantly higher level of neutrophils compared to the control group. However, treatment of mice with resveratrol at a concentration of 20 mg/kg markedly reduced the proportion of neutrophils in the tissues of the paw. The reduction in the population of neutrophils in the tissues of mice paw by votalin was lower compared to 20 mg/kg concentration of resveratrol (Fig. 3).

3.4. Resveratrol treatment inhibits the carrageenan induced higher expression of PGE2 in the micemodel

In the mice model of paw swelling induced by carrageenan administration, the expression of PGE2 was enhanced markedly compared to the control group. However, treatment of the mice with 20 mg/kg doses of resveratrol significantly ($P < 0.02$) reduced the expression of PGE2 in the mice carrageenan administered mice compared to the control group (Fig. 4).

3.5. Resveratrol treatment inhibits the expression of COX-2 in the mice model of edema

Administration of carrageenan markedly enhanced the expression of COX-2 in the tissue samples of mice. On the other hand, resveratrol played a vital role in the inhibition of carrageenan induced increase in the expression of COX-2 in mice. The inhibition in the COX-2 expression by 20 mg/kg doses of resveratrol was significantly higher compared to the known drug, votalin (Fig. 5).

4. Discussion

The present study was performed to investigate the effect of resveratrol on the acute pharyngitis in the mice model of ear swelling and paw swelling in the mice. The study revealed that resveratrol treatment prevents acute pharyngitis in mice model through inhibition of the expression of PGE2 and COX-2 levels.

One of the commonly used animal model for the analysis of the effect of chemotherapeutic agents on ear edema is prepared by the

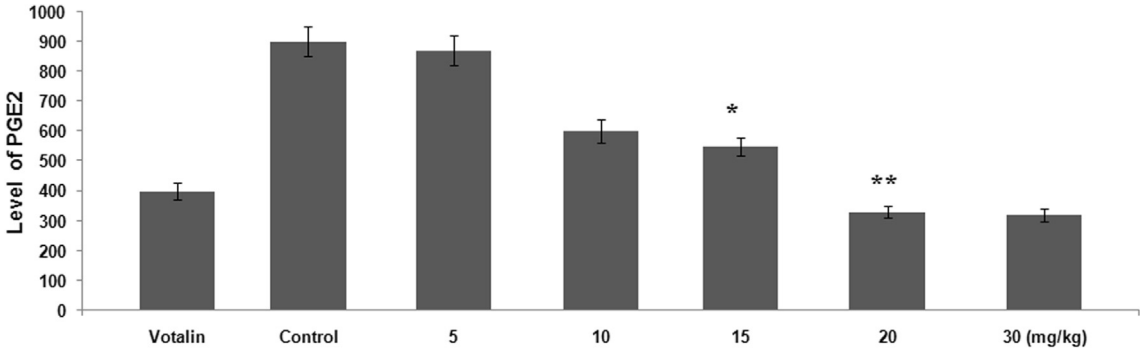

Fig. 4. Inhibition of PGE2 expression in the mice paw homogenates administered with carrageenan by resveratrol treatment. Following administration of carrageenan the mice treated with 5, 10, 15, 20 and 30 mg/kg doses of resveratrol were examined for the expression of prostaglandin E2.

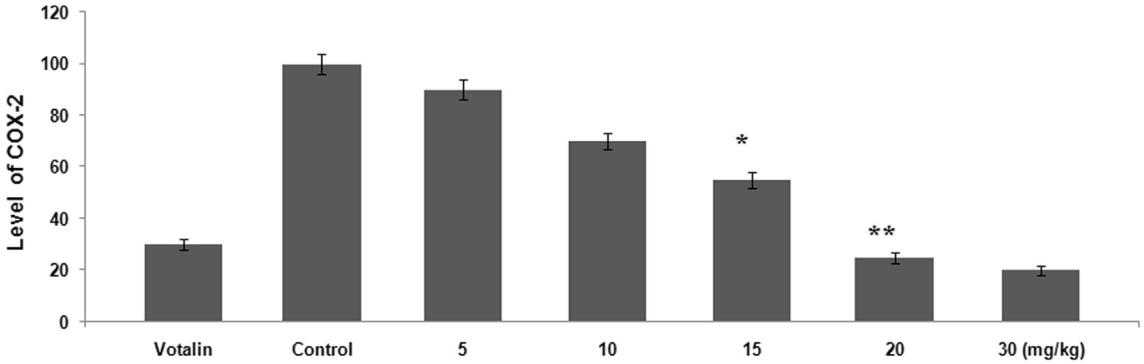

Fig. 5. Inhibition of cyclooxygenase-2 expression in the mice paw homogenates administered with carrageenan by resveratrol treatment. Following administration of carrageenan the mice treated with 5, 10, 15, 20 and 30 mg/kg doses of resveratrol were examined for the expression of cyclooxygenase-2.

administration of xylene to the mice (Zhang et al., 2008; Wang et al., 2012). In the current study administration of xylene to the mice led to ear swelling to a marked extent compared to the control group. Treatment of the mice with resveratrol led to the inhibition of ear swelling significantly compared to the model control group. The effect of resveratrol in the inhibition of ear swelling was more promising compared to the known votalin drug. The mice model of paw swelling is prepared commonly for the study by the administration of carrageenan to the mice (Li et al., 2011; Niu et al., 2012; Valsan and Raphael, 2016; Sreeshma et al., 2016). Among various other factors swelling is caused by the release of prostaglandins (PGs) in the paw tissues. The current study revealed that carrageenan induced paw swelled mice on treatment with resveratrol showed a significant ($P < 0.02$) reduction of paw swelling at 20 mg/kg dose compared to the control group. The inhibition of paw swelling in mice was also caused by votalin by the extent of reduction was significantly ($P < 0.02$) lower compared to the resveratrol treatment. Our study showed that resveratrol treatment significantly reduced the population of inflammatory enhanced by the administration of carrageenan in the tissues of paw. Conversion of arachidonic acid to the prostaglandins is catalyzed by the enzyme known as cyclooxygenase. Alteration in the expression of cyclooxygenase-2 is responsible for the onset of various disorders including, gastrointestinal tract disorder (Botting, 2000; Minghetti, 2004). In the current study carrageenan administration increased the expression of cyclooxygenase-2 and prostaglandins to a considerable extent. However, treatment of the mice with resveratrol significantly inhibited the expression of cyclooxygenase-2 and prostaglandins in the mice paw tissues.

In summary, the current study revealed that resveratrol treatment inhibits acute pharyngitis in the mice model through inhibition of PGE2/COX-2 expression. Thus resveratrol can be used for the treatment of acute pharyngitis.

Conflict of interest

The authors declare that they have no conflicts of interest.

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