Antiasthmatic activity of quercetin glycosides in neonatal asthmatic rats

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Abstract

The present study investigated the anti-asthmatic activity of quercetin glycosides in neonatal asthmatic rats. Rats were divided into four groups: sham (non-asthmatic), asthmatic control, quercetin (25 mg/kg), and quercetin (50 mg/kg). Inflammatory cells in bronchoalveolar lavage fluid (BALF), inflammatory markers, apoptosis, fibrinogen level, prothrombin time, thrombin time, activated partial thromboplastin time, coagulation factor activity, and histopathology were monitored. Quercetin significantly reduced total leukocytes, eosinophils, tumor necrosis factor- α (TNF- α), interleukin (IL-6), nitric oxide (NO), and apoptosis. It also considerably reduced blood coagulation time and coagulation factor activity compared to the controls. The mRNA expression levels of TNF- α , IL-6, and inducible nitric oxide synthase (iNOS) were elevated in asthmatic rats by 1.3-, 1.04-, and 1.1-fold, respectively. However, treatment with 50 mg/kg quercetin glycosides significantly reduced the mRNA expression of TNF- α , IL-6, and iNOS by more than 40%. Quercetin considerably reduced the protein expression of iNOS. Airway and blood vessel narrowing, as well as the accumulation of eosinophils in the lungs were observed in neonatal asthmatic rats. However, treatment with quercetin glycosides significantly reduced inflammation and eosinophil infiltration. In summary, quercetin glycosides significantly attenuated levels of inflammatory markers, demonstrating its protective effects against neonatal asthma in rats.

Keywords Asthma · Inflammation · Neonatal · Quercetin · Rats

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Introduction

Asthma is a well known chronic disease affecting the airways (Martinez 2007). The primary symptoms of asthma include bronchospasm, airflow obstruction, chest tightness, coughing, wheezing, and shortness of breath (Han et al. 2017). Asthma cannot be cured, although symptoms can be addressed with medication (Han et al. 2017). Avoiding smoking, aspirin, and pets are the most effective steps to improve the symptoms of this condition, and drugs are recommended based on the severity of symptoms (Kelly et al. 1988). For temporary relief, bronchodilators are widely recommended (Shichinohe et al. 1996). There are no permanent therapeutic approaches available for the treatment of asthma.

Quercetin is a well known flavonoid that is abundant in vegetables, fruits, grains, leaves, and red onions (Yeh et al. 2016). It is the most abundant flavonoid found in plants (Herrmann 1988; Ross and Kasum 2002). Quercetin is an ingredient in some dietary supplements and has a bitter flavor (Chuang et al. 2016). Several researchers have

reported the use of quercetin in respiratory tract diseases (Di Carlo et al. 1999; Capasso et al. 2003). Many studies have reported various pharmacological and biological activities of quercetin including inhibition of histamine release, angiogenesis, Na^+/K^+ ATPase, angiotensin-converting enzyme II, tyrosine kinase, intestinal peristalsis, and protein kinase C. In addition, quercetin has cell-cycle modulating, antioxidant, antihypertensive, hepatoprotective, anticarcinogenic, and pro-apoptotic effects (Formica and Regelson 1995; Kuo 1997; Duarte et al. 2002; Murota and Terao 2003; Fusi et al. 2003; Gharzouli and Holzer 2004; Janbaz et al. 2004). Helms and Miller (2006) reported the protective effects of quercetin against chronic rhinosinusitis. Therefore, this study investigated the anti-asthmatic activity of quercetin glycosides in neonatal asthmatic rats.

Materials and methods

Materials

Quercetin was obtained from Sigma-Aldrich (Q4951; St. Louis, MO, USA). Anti-iNOS antibody was purchased from Abcam (ab3523; Cambridge, MA, USA). Primers were purchased from Macrogen (Seoul, Korea). Male neonatal Sprague Dawley rats were obtained from the animal house of Huai'an Maternity and Child Health Care Hospital, Huaian, Jiangsu, China. Rats (6–10 g) were divided into four homogeneous groups and were allowed standard access to food and water with a standard light period (12 h of light/dark). Animals were allowed for adaptive feeding 7 days before the experiments. All experimental procedures involving rats were monitored and approved by the Huai'an Maternity and Child Health Care Hospital, Huaian, Jiangsu, China.

Induction of asthma and experimental groups

Experimental asthma was induced in neonatal rats according to a previously described method (Zemmouri et al. 2017). Briefly, rats were sensitized with ovalbumin (1 mg) intraperitoneally adsorbed on $\text{Al}(\text{OH})_3$ gelatinous (20 mg) on 0, 7 and 14 days. Then, rats were challenged with ovalbumin (1.1%) in normal saline (100 μl) through intratracheal instillation on the 21st day. The following groups were used in this study: Group I: sham (normal rats), Group II: control (asthmatic rats), Group III: quercetin (25 mg/kg), and Group IV: quercetin (50 mg/kg). Quercetin was dissolved in dimethyl sulfoxide and administered orally to the neonatal rats for 15 consecutive weeks.

Collection of blood

At the end of treatments, rats were anesthetized by intraperitoneal administration of ketamine (100 mg/kg)/xylazine (10 mg/kg) and sacrificed by decapitation; blood was collected.

Preparation of lung tissue homogenate

After the mice were sacrificed, their lungs were surgically removed, and the tissues were cut into small pieces and homogenized in Tris-HCl buffer (50 mM, pH 7.4) at 10,000 rpm for 10 min. Tissue homogenate was centrifuged, and the supernatant was collected for further experiments (Upreti et al. 1991). All homogenate and supernatant preparations were performed at 4 °C.

Measurement of inflammatory cell numbers

Inflammatory cell counts were determined in bronchoalveolar lavage fluid (BALF) according to a previously described method (Feng et al. 2014).

Determination of serum inflammatory markers

Nitrate and nitrite are final products of NO metabolism and these levels are used for the quantification of NO. Sum of nitrate and nitrite accounts for the total NO concentration. Griess's reaction was used for the determination of NO level in serum based on the nitrite concentration. In the serum, nitrate was reduced to nitrite in the presence of cadmium (Sigma-Aldrich Inc., Shanghai, China). Then, nitrite was converted to nitric acid that reacts with Griess's reagent (Sigma-Aldrich Inc., Shanghai, China) to produce color. Serum level of nitrite was determined by measuring absorbance at 540 nm in a spectrophotometer (Shaheen et al. 2016). Serum levels of tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) were determined according to a previously described method (Tavakkol Afshari et al. 2005).

Determination of blood coagulation time and coagulation factor activity

Fibrinogen level (FIB), prothrombin time (PT), thrombin time (TT), activated partial thromboplastin time (APTT), and the activity of coagulation factors were determined according to previously described methods (Feng et al. 2014).

Determination of apoptosis

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and staining were performed in lung sections as previously described (Sheikh-Hamad et al. 2004). Briefly,

lung tissues were excised and perfused in normal saline, and then fixed in 10% neutral formalin (10%) for 24 h. Next, the lung sections were dehydrated with a graded series of alcohol and embedded in paraffin film. Finally, the sections were cut (4–5 µm thick) with a rotary microtome, and TUNEL staining was performed as previously described (Fayzullina and Martin 2014).

Determination of mRNA expression

Total RNA was isolated from neonatal lung tissues using TRIzol reagent, and 20 µl total RNA was converted into cDNA using oligo(dT)₁₈. Quantitative PCR (qPCR) was conducted with cDNA samples using SYBR Green (Sigma). The mRNA expression levels of TNF-α, IL-6, and inducible nitric oxide synthase (iNOS) were determined according to a previously described method (Alfonso et al. 2002). The primers used in this study are shown in Table 1. GAPDH was used as an internal expression control.

Immunohistochemistry

Lung tissues were excised and perfused in normal saline, and then, lung tissues were fixed in 10% neutral formalin (10%) for 24 h. Lung sections were dehydrated by graded alcohol and embedded in paraffin film. Paraffin-embedded lung sections were dewaxed, washed with deionized water and rinsed with PBS. Lung sections were incubated with goat serum (0.5% bovine serum albumin and 0.1% Tween) for 30 min. Then, sections were incubated with the anti-iNOS primary antibody (ab3523; Abcam) at 4 °C for 12 h, followed by the goat anti-mouse secondary antibody (ab205719; Abcam). Then, sections were counterstained with hematoxylin for 60 s. Finally, sections were imaged, and expressions were quantified (Rehg et al. 2012).

Histopathological analyses

Lung tissues were fixed in neutral formalin (10%), and sections 4–5-µm thick were stained with hematoxylin and eosin (H&E) for microscopic analyses (Althnaian et al. 2013).

Statistical analyses

Values are given as means with standard deviations. One-way ANOVA (SPSS 17, IBM SPSS Statistics, Hong Kong) was applied for statistical analyses of data, and Tukey's post hoc tests were used for multiple comparisons. *P* values < 0.05 were considered statistically significant.

Results

This study evaluated the anti-asthmatic activity of quercetin glycosides in neonatal asthmatic rats. Total leukocyte content was substantially increased in neonatal asthmatic rats (Group II) compared to non-asthmatic controls (Group I). Total leukocyte content was increased by 477.1% in asthmatic rats, while quercetin glycoside treatment significantly reduced leukocyte contents by 36.6% and 67.3% in Groups III and IV, respectively (Fig. 1a, *P* < 0.05). Eosinophil content was also substantially increased in asthmatic rats, and quercetin glycosides significantly reduced eosinophils by 33.3% and 70.6% in Groups III and IV, respectively (Fig. 1a, *P* < 0.05). TNF-α and IL-6 levels, as pg/mg protein, were increased by more than 100% in the neonatal asthmatic rats. Treatment of rats with quercetin glycosides significantly reduced TNF-α levels by 32.5% and 73.7% in Groups III and IV, respectively (Fig. 1b, *P* < 0.05), and also reduced IL-6 (Fig. 1b, *P* < 0.05). NO concentration was increased by more than 100% in the neonatal asthmatic rats, and quercetin glycosides significantly reduced levels by 34.7% and 71.7% in Groups III and IV, respectively (Fig. 2a, *P* < 0.05).

FIB was increased by 78.3% in neonatal asthmatic rats. However, treatment of rats with quercetin glycosides significantly reduced FIB by 7.3% and 26.8% in Groups III and IV, respectively (Fig. 2b, *P* < 0.05). PT, TT, and APTT were significantly increased in neonatal asthmatic rats but were considerably reduced following quercetin glycoside supplementation (Fig. 3, *P* < 0.05). The blood coagulation factors FII, FV, FVII, and FX were increased in neonatal asthmatic rats and were also reduced following quercetin glycoside supplementation (Fig. 4, *P* < 0.05).

The percentage of apoptotic cells was elevated in the asthmatic rats. Again, treatment with quercetin glycosides

Table 1 List of RT-PCR primers used for the amplification of TNF-α, IL-6 and iNOS

S. no.	Gene name	Forward	Reverse
1	TNF-α	5'-CCCAGACCCTCACACTCAGAT-3'	5'-TTG TCC CTTGAA GAG AAC CTG-3'
2	IL-6	5'-AAGTTTCTCTCCGCAAGATAC TTCCAG CCA-3'	5'-AGG CAAATTTCTGTTATATCCA GTTT-3'
3	iNOS	5'-CTCCATGACTCTCAGCACAGAG-3'	5'-GCACCGAAGATATCCTCATGAT-3'
4	GAPDH	5'-TCCCTCAAGATTGTCTAGCAA-3'	5'-AGATCCACAACGGATACATT-3'

Fig. 1 Effects of quercetin glycosides on inflammatory cell recruitment in BALF from neonatal asthmatic rats (a). Effects of quercetin glycosides on TNF- α and IL-6 levels in neonatal asthmatic rats expressed as pg/mg protein (b). * $P < 0.05$ vs. Group I and # $P < 0.05$ vs. Group II

significantly reduced apoptosis by 39.7% and 72.7% in Groups III and IV, respectively (Fig. 5, $P < 0.05$). The mRNA expression of inflammatory markers, such as TNF- α , IL-6, and iNOS, was quantified and expressed as fold changes. TNF- α , IL-6, and iNOS expression were increased by 1.3-, 1.04- and 1.1-fold, respectively, in the asthmatic group. However, treatment of rats with quercetin glycosides significantly reduced expression of all three markers by more than 0.4-fold in Group IV (Fig. 6, $P < 0.05$). Immunohistochemistry showed that iNOS protein expression increased by 0.9-fold in the asthmatic rats. Quercetin treatment reduced iNOS protein levels by 15.3% and 35.3% in Groups III and IV, respectively (Fig. 7, $P < 0.05$). Airway and blood vessel narrowing and the accumulation of eosinophils was observed in the lungs of neonatal asthmatic rats. However, treatment of rats with quercetin glycosides significantly reduced inflammation and eosinophil infiltration of the lung (Fig. 8).

Discussion

We evaluated the anti-asthmatic activity of quercetin glycosides in neonatal asthmatic rats. Several researchers have reported the wide usage of quercetin for the treatment of

respiratory tract diseases (Di Carlo et al. 1999; Capasso et al. 2003). The various pharmacological and biological activities of quercetin such as inhibition of histamine release, and effects on angiogenesis, the Na⁺/K⁺ ATPase, angiotensin-converting enzyme II, tyrosine kinase, intestinal peristalsis, and protein kinase C have also been demonstrated (Murota and Terao 2003). In addition, quercetin affects the cell cycle and has antioxidant, antihypertensive, hepatoprotective, anti-carcinogenic, and pro-apoptotic effects (Formica and Regelson 1995; Kuo 1997; Duarte et al. 2002; Murota and Terao 2003; Fusi et al. 2003; Gharzouli and Holzer 2004; Janbaz et al. 2004). Helms and Miller (2006) reported the protective effects of quercetin against chronic rhinosinusitis.

NO is a vital messenger that participates in various types of pathophysiology and plays a crucial role in the inhibition of non-adrenergic transmission (Gross et al. 1991; Ellis and Undem 1992; Fischer et al. 1993). Researchers have reported that NO is abundant in airway respiratory epithelia (Folkerts and Nijkamp 1998; Bove and van der Vliet 2006). Researcher have attributed increases in NO generation to the increased production of cytokines, iNOS, and prostaglandins. Increased NO production leads to oxidative stress, DNA damage, and cell injury (Murphy 1999). In our study, NO production was significantly reduced following

Fig. 2 Effects of quercetin glycosides on NO levels (mM) in neonatal asthmatic rats (a). Effects of quercetin glycosides on FIB levels (g/l) in neonatal asthmatic rats (b). * $P < 0.05$ vs. Group I and # $P < 0.05$ vs. Group II

Fig. 3 Effects of quercetin glycosides on TT, PT, and APTT in neonatal asthmatic rats; all times are in seconds (s). * $P < 0.05$ vs. Group I and # $P < 0.05$ vs. Group II

Fig. 4 Effects of quercetin glycosides on active blood coagulation factors FII, FV, FVII, and FX in neonatal asthmatic rats. Values indicate time in seconds (s). * $P < 0.05$ vs. Group I and # $P < 0.05$ vs. Group II

Fig. 5 Effects of quercetin glycosides on apoptosis in neonatal asthmatic rats. Values represent the percentage of cells in apoptosis. * $P < 0.05$ vs. Group I and # $P < 0.05$ vs. Group II

quercetin supplementation. Our findings are consistent with earlier studies reporting quercetin-induced inhibition of NO (Gharzouli and Holzer 2004; Roghani et al. 2004; Ajay et al. 2003). Jackson and Venema (2006) found that quercetin inhibited NO synthase in endothelial cells of the bovine aorta.

Ko et al. (2002) reported the antispasmodic effects of 3-*O*-methyl quercetin (a quercetin glycoside) in isolated guinea pig trachea. Asthma involves chronic airway inflammation, and its significant symptoms include coughing, wheezing, and chest tightness. The infiltration of

eosinophils, lymphocytes, and mast cells into the airway wall leads to mucus hypersecretion and allergic inflammation. Lung histopathological analyses confirmed the presence of severe inflammation in rats with induced asthma. Boots et al. (2011) reported that quercetin significantly decreased inflammation and oxidative stress in sarcoidosis. Askari et al. (2012) found that supplementation of quercetin with vitamin C also decreased inflammation and oxidative stress. In this study, the treatment of rats with quercetin glycosides significantly attenuated inflammation, which indicates its protective effect against asthma.

Fig. 6 Effects of quercetin glycosides on the mRNA expression levels of TNF- α , IL-6, and iNOS in neonatal asthmatic rats. * $P < 0.05$ vs. Group I and # $P < 0.05$ vs. Group II

Fig. 7 Effects of quercetin glycosides on iNOS protein expression in neonatal asthmatic rats. * $P < 0.05$ vs. Group I and # $P < 0.05$ vs. Group II; scale bar is 100 μ m

Fig. 8 Effects of quercetin glycosides on the cellular architecture of lungs in neonatal asthmatic rats. Sections were stained with H&E and analyzed by microscopy. Magnification = 100×; $n = 6$

Conclusion

In summary, quercetin glycosides significantly reduced inflammatory markers, which suggests that they have protective effects against neonatal asthma in rats.

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Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest.

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