



Available online at
 ScienceDirect
www.sciencedirect.com

Elsevier Masson France

www.em-consulte.com



Original article

The efficiency of polyphenolic compounds on allergen induced hyperreactivity of the airways

S. Franova^{a,*}, M. Joskova^a, M. Sutovska^a, E. Novakova^b, K. Adamicova^c, O. Pechanova^d, G. Nosalova^a

^a Department of pharmacology, Jessenius faculty of medicine, Comenius University, Sklabinska 26, 037 53 Martin, Slovakia

^b Department of microbiology and immunology, Jessenius faculty of medicine, Comenius University, Martin, Slovakia

^c Department of pathological anatomy, Jessenius faculty of medicine, Comenius University, Martin, Slovakia

^d Department of normal and pathological physiology, Slovak Academy of Sciences, Bratislava, Slovakia

ARTICLE INFO

Article history:

Received 28 July 2010

Accepted 30 September 2010

Keywords:

Airways hyperresponsiveness

Allergic inflammation

Polyphenols

ABSTRACT

The results of some epidemiologic studies have suggested that the consumption of the food containing polyphenolic compounds might reduce the occurrence of asthma symptoms. The aim of our experiments was to evaluate the effect of the 21-day red-wine polyphenolic compounds (Provinol) administration on allergen (OVA) induced inflammation of the airways in experimental conditions. The allergic inflammation of the airways was induced in guinea pigs by 21-day ovalbumin sensitization. The tracheal smooth muscle reactivity was examined by in vitro method to bronchoconstricting mediator histamine (10^{-8} – 10^{-3} mol/L); the changes in airways resistance were examined by in vivo method by exposure to nebulized histamine (10^{-6} mol/L). The histological investigation of the tracheal tissue and BALF levels of IL-4, IL-5 were used as parameters of the airway inflammation. A 21-day administration of Provinol caused a significant decrease of the specific airway resistance after the histamine nebulization and the decline in tracheal smooth muscle contraction amplitude to this bronchoconstrictor. Provinol minimized the degree of inflammation estimated on the basis of eosinophil calculation and levels of inflammatory cytokines IL-4 and IL-5. In conclusion, the administration of Provinol leads to bronchodilating and anti-inflammatory effects in ovalbumin-sensitized guinea pigs, suggesting their possible use in the therapy of allergic inflammatory conditions of the airways.

© 2010 Elsevier Masson SAS. All rights reserved.

1. Introduction

Various epidemiological studies have indicated that the consumption of foods rich in polyphenols, including those contained in fruits or vegetables, is associated with the lower incidence of asthma symptoms [1]. Polyphenolic compounds (flavonoids, stilbenes, phenolic acids and others) possess different pharmacodynamic activities. The beneficial effects of polyphenolic compounds might be explained by their actions on a wide range of biological activities affecting mammalian metabolism. Polyphenols are well recognized to possess antioxidant and free radical scavenging properties, they were also reported to affect the activity of enzymes critically involved in pathways that regulate cell proliferation and immune cell activation.

In respiratory tract, polyphenols have antiallergic [2], anti-inflammatory [3], and bronchodilatory effect [4], which might explain reports suggesting their beneficial effects in asthma. Some members of flavonoid group are able to inhibit during asthmatic

inflammation the release and synthesis of specific bronchoconstricting mediators [5], to ameliorate the increase in inflammatory cytokines, chemokines, eosinophils and allergen specific antibodies [2,3]. Asthma severity was less common in adults who consumed apples and a moderate amount of red wine rich in polyphenolic compounds [6].

In previous studies, we have reported that dry powder of polyphenolic compounds from red wine (RPWc, Provinol) is able to produce ex-vivo relaxation of tracheal strips in guinea pigs sensitized with allergen and to influence the metabolism of NO participating in its bronchodilatory effect. Provinol inhibited histamine, acetylcholine induced contraction of tracheal smooth muscle in guinea pigs with ovalbumin induced hyperreactivity of the airways [7].

For that reason, the aim of the present study was to evaluate the complete effect of long-term RPWc administration on airways reactivity and the degree of inflammation during experimentally induced chronic allergic inflammation. In view of this fact, our experiments were focused on the evaluation of 21 days Provinol administration efficiency: on tracheal smooth muscle reactivity in ovalbumin sensitised guinea pigs investigated by in vitro method; on changes in specific airway resistance to histamine measured by

* Corresponding author. Tel.: +421 43 4132535; fax: 421 43 4134807.

E-mail address: franova@jfm.uniba.sk (S. Franova).

in vivo method; on the degree of inflammation evaluated by eosinophil calculation and the estimation of inflammatory cytokines IL-4, IL-5 in bronchoalveolar lavage fluid (BALF).

2. Materials and methods

Guinea pigs (200–250 g) were randomly divided into three experimental groups:

- group 1: (Control) treated 21 days with saline only;
- group 2: sensitized 21 days with OVA;
- group 3: OVA sensitized and simultaneously treated 21 days with Provinol (20 mg/kg per day).

The dose of RWPC corresponded to 10 times the concentration that produced maximal relaxation of endothelial smooth muscle. Because only 5 to 10% of polyphenols could be absorbed in the digestive tract [8].

The number of animals in each experimental group was 10. The experimental study was approved by the Ethical Committee of Jessenius Faculty of Medicine in Martin.

3. Material

Dry powder of red wine polyphenolic compounds (Provinol) was provided by D. Ageron (*Société française de Distillerie, Vallont Pont d'Arc, France*). The composition of Provinol was (in mg/1 g of dry powder): proanthocyanins 480, total anthocyanins 61, free anthocyanins 19, catechin 38, hydroxycinnamic acid 18, flavonols 14 [9].

Ovalbumin (OVA, egg albumin grade V), histamine (histamine-2HCl) and other chemicals were purchased from Sigma Chemicals Co, Germany.

Mouse IL-5, IL-4 Elisa Kits for protein array systems were purchased from Ray Biotech, Inc.

4. Sensitization of guinea pigs

Male Trik guinea pigs (200–250 g) were actively sensitized by administration of ovalbumin (OVA; Grade V, Sigma). Five milligram of OVA (OVA with 1 mg of aluminium hydroxide) was injected intraperitoneally together with 5 mg subcutaneous OVA injection in 1 ml of saline on day 1. Subsequently, guinea pigs were treated 21 days with repeated i.p. OVA injections every 3 days. Further guinea pigs were exposed to nebulized OVA (1% in 0.9% sterile sodium chloride solution) in an aerosol chamber last 5 days. This scheme of allergen sensitization in guinea pigs airways induced a sufficient degree of chronic inflammation [10].

5. In vitro tracheal muscle contraction experiments

The reactivity of the tracheal smooth muscle was estimated by in vitro method, after 21 days' ovalbumin sensitization. The preparation of guinea pig tracheal strips were placed into 20-ml organ chamber containing Krebs-Henseleit buffer of the following composition (μM): NaCl, 110.0; KCl, 4.8; CaCl_2 , 2.35; MgSO_4 , 1.20; KH_2PO_4 , 1.20; NaHCO_3 , 25.0; in glass-distilled water. Organ chambers were maintained at $36.5 \pm 0.5^\circ\text{C}$ and were aerated continuously with the mixture 95% O_2 and 5% CO_2 , to maintain $\text{pH} = 7.5 \pm 0.1$. The tissue strips were initially set to 4 g of tension (30-minute loading phase). After this period, the tension in each tissue segment was readjusted to a baseline of 2 g (30 minutes adaptation phase). The amplitude of contraction (mN) of the tracheal smooth muscle to the cumulative doses of histamine (10^{-8} – 10^{-3} mol/L) was used as a parameter of tracheal smooth muscle reactivity.

6. In vivo evaluation of airway reactivity

In vivo airway reactivity was evaluated using a double chamber bodyplethysmograph (Rodents Double-Chamber Plethysmograph, Hugo Sachs Elektronik-Harvard Apparatus, type 855 with HSE Pulmodyn Pennock W software). The changes in specific airway resistance (RxW) after a short-term inhalation (2 minutes) of bronchoconstricting mediator (histamine: 10^{-6} mol/L in saline) were considered as an indicator of airways reactivity. There was an interval of 5 minutes between histamine exposures and airways resistance measurement. During this interval, fresh air was insufflated into the breathing chamber [11].

7. Measurement of inflammatory cytokines

The left lung was lavaged with 0.9% NaCl (37°C) 2×10 mL/kg and BALF was centrifuged at 1500 rpm for 10 minutes. The supernatant from the lavage was stored for subsequent biological analysis.

The levels of IL-4, IL-5 were determined by Elisa method in BALF by using commercially available kits (Ray Biotech, Inc.). The minimum detectable level of cytokines was less than 1 pg/mL.

8. Histological examination

The trachea was fixed in 10% buffered formaldehyde. The sections of 10 μm wide were then stained by standard method with haematoxylin and eosin for light microscopy examination (enlargement: 240 times). On the basis of eosinophil amounts the degree of inflammation was divided into mild, medium, strong (mild: up to 20; medium: up to 50; strong: over 50).

9. Data analysis

The statistical analysis was performed using Anova test. Differences were considered statistically significant when P -value was below 0.05. All results are expressed as mean $\pm$ SEM.

10. Results

Three weeks after sensitization of guinea pigs and simultaneous RWPC administration the following parameters were investigated: the amplitude of tracheal smooth muscle strips contraction by in vitro method; the changes in specific airways resistance by in vivo method; the amount of eosinophils in tracheal tissue; the levels of inflammatory cytokines typical for allergic inflammation IL-4, IL-5 in bronchoalveolar lavage fluid (BALF).

The effect of Provinol on the tracheal smooth muscle reactivity in OVA sensitised guinea pigs was evaluated after 21 days of administration by in vitro method to cumulative doses of bronchoconstricting mediator histamine (10^{-8} – 10^{-3} mol/L). Long-lasting treatment of experimental animals with Provinol inhibited the contraction induced by cumulative doses of histamine (Fig. 1).

Specific airway resistance measured in the double-chamber bodyplethysmograph was used as a marker of in vivo airway reactivity. Twenty-one-day administration of Provinol caused a significant decrease of specific airway resistance (RxW) after histamine (10^{-6} mol/L) nebulization (Fig. 2).

Except for modulation of OVA-potentiated airway bronchoconstriction and bronchial hyperreactivity, Provinol administered during 21 days at a daily dose of 20 mg/kg also reduced the levels of inflammatory cytokines typical for allergic inflammation of the airways: IL-4 (Fig. 3) and IL-5 (Fig. 4) in BALF.

Histological examination of tracheal mucosa of guinea pig after ovalbumin sensitization revealed eosinophilic infiltration of

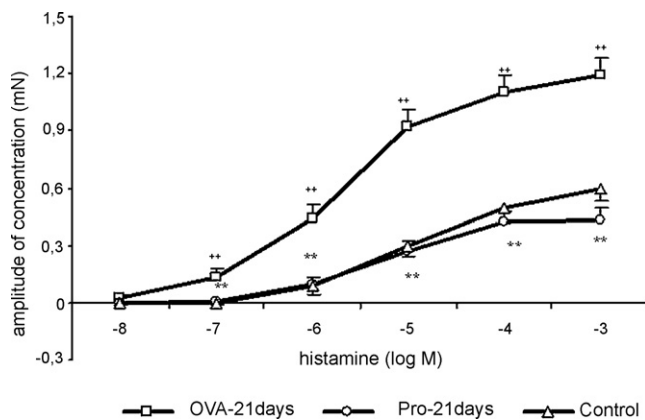


Fig. 1. Changes in the amplitude of tracheal smooth muscle contraction to cumulative doses of histamine after 21-day Provinol administration in guinea pigs sensitized 21 days with ovalbumin (OVA). Δ : Control group; $\square$: guinea pigs after 21-day ovalbumin sensitization; $\circ$: sensitized guinea pigs treated 21 days with Provinol; data are expressed as the mean $\pm$ SEM; $n = 12$ for each group; statistically significant difference $*P < 0.05$ and $**P < 0.01$ control group versus OVA; $*P < 0.05$ and $**P < 0.01$ OVA versus OVA with Provinol.

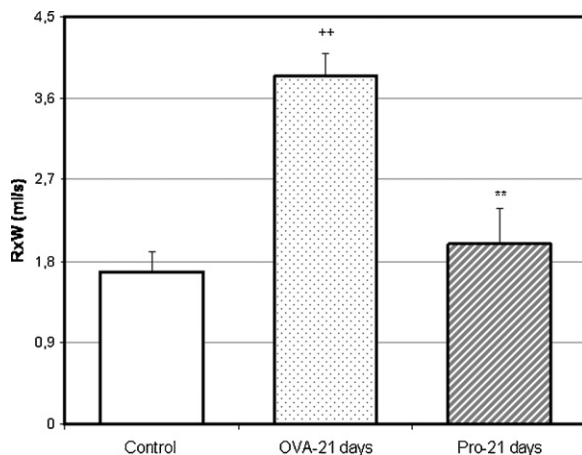


Fig. 2. Comparison of specific airway resistance after inhalation of histamine (10^{-6} mol/l) between control (healthy guinea pigs); 21 days OVA sensitized (OVA-21 days); and OVA – sensitized treated 21 days with Provinol (Pro-21 days). Data are expressed as the mean $\pm$ SEM; $n = 12$ for each group; statistically significant difference $**P < 0.01$ control group versus OVA; $**P < 0.01$ OVA versus OVA with Provinol.

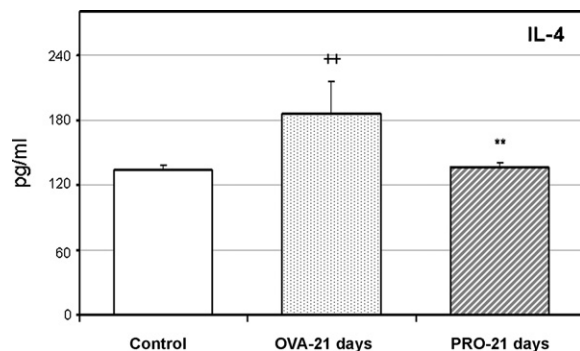


Fig. 3. Comparison of inflammatory cytokines IL-4 levels in BALF between control (healthy guinea pigs); 21 days OVA sensitized (OVA-21 days); and OVA – sensitized treated 21 days with Provinol (Pro-21 days). Data are expressed as the mean $\pm$ SEM; $n = 12$ for each group; statistically significant difference $**P < 0.01$ control group versus OVA; $**P < 0.01$ OVA versus OVA with Provinol.

epithelium and submucosa (found degree of inflammation in 10 experimental animals: 3 $\times$ mild, 4 $\times$ medium, 1 strong). Eosinophils were formed into micro-abscess in the epithelium. Long-term administration of Provinol caused a diminution of

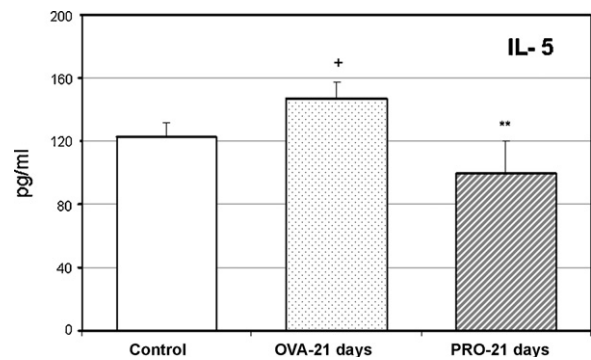


Fig. 4. Comparison of inflammatory cytokines IL-5 levels in BALF between control (healthy guinea pigs); 21 days OVA sensitized (OVA-21 days); and OVA – sensitized treated 21 days with Provinol (Pro-21 days). Data are expressed as the mean $\pm$ SEM; $n = 12$ for each group; statistically significant difference $*P < 0.05$ control group versus OVA; $**P < 0.01$ OVA versus OVA with Provinol.

eosinophilic infiltration (found degree of inflammation in 10 experimental animals: 2 $\times$ mild).

11. Discussion and conclusion

The present study shows that 21 days oral administration of RWPC (Provinol) has the apparent efficacy on parameters of experimentally induced allergic inflammation of the airways. Results of our experiments confirmed that the 21-day administration of RWPC has a positive effect in modulation of tracheal smooth muscle reactivity estimated by in vitro and in vivo method. Except for bronchodilatory activity dry powder of red wine polyphenolic compounds is able to minimize the airways inflammation induced by ovalbumine sensitization in experimental animals.

Allergic asthma is a chronic inflammatory disease of the airways. Characteristic features of allergic asthma are allergen-induced early and late bronchial obstructive reactions, airway inflammation, structural changes of the airway wall associated with progressive decline in lung function, and airway hyperresponsiveness (AHR). AHR is defined by an exaggerated obstructive response of the airways to a variety of pharmacological, chemical and physical stimuli [12].

We confirmed that Provinol is able to minimize tracheal smooth muscle hyperreactivity. Provinol decreased the amplitude contraction of the tracheal smooth muscle strips (prepared from OVA sensitized guinea pigs) to cumulative doses of histamine. Furthermore, long-lasting administration of Provinol caused a significant decrease of specific airway resistance measured in a body double chamber bodyplethysmograph after histamine nebulization.

In addition to smooth muscle hyperreactivity allergic asthma is associated with ongoing airway inflammation. Inflammation is thought to cause symptoms of asthma directly and indirectly by inducing contraction of airway smooth muscle, enhancing airway responsiveness to various stimuli and by inducing changes in structural components of the airway wall leading to airway remodelling [13].

In allergic asthma, it is assumed that the early-phase reaction is based on an immunoglobulin IgE mediated reaction with antigen cross-linking of IgE molecules bound to specific receptors on mast cells. Subsequent activation of IgE (Fc ϵ R1 receptors) leads to the release of bronchoconstricting mediators including mainly leukotrienes and histamine. The allergic late-phase reaction is in addition to previous effects links with infiltration of the airway wall by inflammatory cells (eosinophils, lymphocytes, especially CD4-positive T-cells). The immunological processes involved are characterized by proliferation and activation of Th2 lymphocytes

[14]. Th2-derived cytokines play a key role in orchestrating the eosinophilic inflammatory response in asthma. The Th2 mediated allergic inflammation is accomplished with cytokines such as IL-4 and IL-13 for their role in directing B lymphocytes to synthesize IgE, IL-5 for its role in maturation and activation of eosinophils, and IL-13 for its role in mast cell genesis [15]. The estimation of IL-4, IL-5 and other inflammatory cytokines is good diagnostic marker characterised the degree of asthmatic inflammation.

Long-lasting administration of Provinol showed its ability to ameliorate the levels of inflammatory cytokines IL-4 and IL-5 in BALF of sensitized experimental animals and concurrently decreased the degree of inflammation estimated on the basis of eosinophil calculation.

Provinol, a red wine polyphenolic compounds, acts as an antioxidant, it enhances cNO concentration, positively modulates the process of thrombosis, and has cardioprotective, antihypertensive, antiischemic and other positive effects [9].

Our present results demonstrate that Provinol with the exception of bronchodilatory effect has antiinflammatory properties and this outcome confirm the hypothesis that may become an additional therapy in prevention of airway hyperresponsiveness in asthma subjects.

Conflict of interest statement

The authors declared no conflict of interest in relation to this article.

Acknowledgement

This work was supported by Center of experimental and clinical respirology – “Project co-financed from EC sources” – ERDF: European Regional Development Fund and Grant VEGA 1/0073/08.

References

- [1] Varraso R, Kauffmann F, Leynaert B, Boutron-Ruault MC, Clavel-Chapelon F, Romieu I. Dietary patterns and asthma in the E3N study. *Eur Respir J* 2009;33:33–41.
- [2] Sanbongi C, Takano H, Osakabe N, Sasa N, Natsume M, Yanagisawa R, et al. Rosmarinic acid in perilla extract inhibits allergic inflammation induced by mite allergen, in a mouse model. *Clin Exp Allergy* 2004;34: 971–7.
- [3] Rogerio AP, Kanashiro A, Fontanari C, Da Silva EVG, Lucisano-Valim YM, Soares EG, et al. Anti-inflammatory activity of quercetin and isoquercitrin in experimental murine allergic asthma. *Inflamm Res* 2007;56:402–8.
- [4] Das M, Ram A, Ghosh B. Luteolin alleviates bronchoconstriction and airway hyperreactivity in ovalbumin sensitized mice. *Inflamm Res* 2003;52: 101–6.
- [5] Fernandez J, Reyes R, Ponce H. Isoquercitrin from Argemone platyceras inhibits carbachol and leukotriene D4-induced contraction in guinea pigs airways. *Eur J Pharmacol* 2005;522:108–15.
- [6] Garcia V, Arts ICW, Sterne JAC, Thompson RL, Shaheen SO. Dietary intake of flavonoids and asthma in adults. *Eur Respir J* 2005;26:449–52.
- [7] Franova S, Nosalova G, Pechanova O, Sutovska M. Red wine polyphenolic compounds inhibit tracheal smooth muscle contraction during allergen-induced hyperreactivity of the airways. *J Pharm Pharmacol* 2007;59: 727–32.
- [8] Diebolt M, Bucher B, Andriantsitohaina R. Wine polyphenols decrease blood pressure, improve NO vasodilatation and induce gene expression. *Hypertension* 2001;38:159–65.
- [9] Zenebe W, Pechanova O. Red wine polyphenols induce vasorelaxation by increase nitric oxide activity. *Physiol Res* 2003;52:425–32.
- [10] Franova S, Joskova M, Novakova E, Adamcova K, Sutovska M, Nosal S. Effects of flavin 7 on allergen induced hyperreactivity of airways. *Eur J Med Res* 2009;7:78–81.
- [11] Mokry J, Mokra D, Nosalova G, Beharkova M, Feherova Z. Influence of selective inhibitors of phosphodiesterase 3 and 4 on cough and airway reactivity. *J Physiol Pharmacol* 2008;59(Suppl. 6):473–82.
- [12] Meurs H, Gosens R, Zaagsma J. Airway hyperresponsiveness in asthma: lessons from in vitro model systems and animal models. *Eur Respir J* 2008;32:487–502.
- [13] Zuyderduyn S, Sukkar MB, Fust A, Dhaliwal S, Burgess JK. Treating asthma means treating airway smooth muscle cells. *Eur Respir J* 2008;32:265–74.
- [14] Van Oosterhout JM, Bloksma N. Regulatory T-lymphocytes in asthma. *Eur Respir J* 2005;26:918–32.
- [15] Kips JC. Cytokines in asthma. *Eur Respir J* 2001;18:24–33.