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Red wine polyphenolic compounds inhibit tracheal smooth muscle contraction during allergen-induced hyperreactivity of the airways

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Abstract

The aims of the study were to investigate the short and long-term effects of Provinol (red wine polyphenolic compounds) on tracheal smooth muscle reactivity using an in-vitro model of ovalbumin-induced airway inflammation in guinea-pig trachea, and to evaluate the role of nitric oxide (NO) in the bronchodilatory effect of Provinol.

The amplitude of tracheal smooth muscle contraction in response to mediators of bronchoconstriction – histamine (10 nM–1 mM), acetylcholine (10 nM–1 mM) and to allergen (ovalbumin 10^{-5}–10^{-3} g mL^{-1}) was used as a parameter of tracheal smooth muscle reactivity. To test the short-term effects of Provinol, isolated tracheal strips were pre-treated for 30 min with Provinol (10^{-4} mg mL^{-1}) alone or in combination with N^{ω}-nitro-L-arginine methyl ester (L-NAME; 10^{-6} mol L^{-1}). To test the long-term effects of Provinol, isolated tracheal strips were prepared from guinea pigs that had been treated for 14 days with Provinol (20 mg kg^{-1} per day) alone or in combination with L-NAME (40 mg kg^{-1} per day).

Incubation of tracheal smooth muscle with Provinol decreased the amplitude of contraction in response to ovalbumin, histamine and acetylcholine. The non-selective NO synthase inhibitor L-NAME partially abolished the effect of Provinol on acetylcholine and ovalbumin-induced but not histamine-induced bronchoconstriction. A similar profile was observed after 14 days' oral administration of Provinol.

In conclusion, Provinol inhibited the allergen- and spasmogen-induced contraction of tracheal smooth muscle in ovalbumin-sensitized guinea pigs via a mechanism that was mediated at least partially through the metabolism of NO.

Introduction

Epidemiological studies have suggested that dietary factors, including the consumption of food that contains polyphenolic compounds, might reduce the occurrence of asthma symptoms. Asthma symptoms were less severe in adults who consumed more apples and a moderate amount of red wine (Shaheen et al 2001). In-vitro studies have confirmed that the beneficial effect of fruit and red wine may be partly explained by the presence of polyphenols. Indeed, polyphenols have been shown to have several biological activities, including antioxidant, anti-inflammatory, bronchodilatory and anticarcinogenic properties. Sanbongi et al (2004) confirmed that oral administration of the polyphenolic phytochemical rosmarinic acid inhibited an allergic reaction in mice, possibly by ameliorating increases in cytokines, chemokines and allergen-specific antibodies. Furthermore, one particular flavonoid, khellin, known for its bronchodilatory properties, was used historically to treat asthma (Kennedy and Stock 1952). Recently, polyphenols of the catechin group, found in green tea, were shown to inhibit production of nitric oxide (NO) and inducible nitric oxide synthase (NOS) gene expression in macrophages by attenuating activation of nuclear factor-κB (NF-κB) activation (Lin and Lin 1997).

In the respiratory tract, NO is derived from the amino acid L-arginine, in a stereospecific reaction catalysed by three isoforms of NOS that differ in activity and tissue distribution. Endothelial (eNOS or NOS-III) is constitutively expressed in endothelial cells (Shaul 2002), bronchial epithelium and in the cells of the basal membrane of ciliary microtubules (Xue et al 1996). NO produced by eNOS regulates airway tone in the respiratory tract, gas

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exchange, function of surfactant and mucocilliary clearance (Bennet et al 1994). Neuronal (nNOS or NOS-I) is localized in both human and animal airway nerve fibres; NO produced in this way is the major mediator of nerve-mediated smooth muscle relaxation (Ricciardolo et al 2004). Expression of inducible NOS (iNOS or NOS-II) has been described in macrophages, neutrophils, and epithelial, endothelial and smooth muscle cells. This isoform is regulated at a pretranslational level and can be induced by allergens, bacterial lipopolysaccharides and pro-inflammatory cytokines such as tumour necrosis factor-α, interferon-γ and interleukin-1β (Sade and Kivity 2002). NO produced by iNOS can exert beneficial effects in the defence of the airways, but large quantities (nanomolar concentrations) can be pro-inflammatory and have detrimental effects (Pitt and St Croix 2002). The detrimental effects are produced because persistent high levels of NO can react with concomitantly produced superoxide ions (Hanacek et al 1991), generating highly toxic compounds such as peroxynitrite and hydroxyl radicals (Weinberger et al 1999).

Some pathological conditions, such as allergen-induced bronchial asthma, affect the physiological equilibrium of NO, either by decreasing production of cNOS-derived NO or by increasing formation of NO by iNOS (Miura et al 1997). This imbalance results in deficiency of the local bronchoprotective functions of NO, which leads to hyperreactivity of the airways and potentiation of inflammation (Alving et al 1993).

The body is probably equipped with a mechanism that regulates the expression of iNOS, and physiological levels of NO may inhibit the activity of transcription factor NF-κB and thus decrease the expression of the iNOS gene (Colasanti and Suzuki 2000). High doses of corticosteroids have been shown to stimulate cNOS activity by suppressing iNOS expression (Riccardiolo 2003) and thus may be able to regulate the balance of NOS activity, which would decrease the hyperreactivity of the respiratory tract during the inflammatory process. Substances that stimulate constitutive NO formation may be able to inhibit airway hyperreactivity, although long-term inhibition of iNOS activity by selective inhibitors did not offer clear clinical benefit in patients with asthma (Keller et al 2005).

In previous experiments, Provinol (a mixture of polyphenolic compounds isolated from red wine) was shown to elicit endothelium-dependent vascular relaxation by stimulation of cNOS activity concomitantly with the scavenging of oxygen free radicals, which together enhance the constitutive NO concentration (Zenebe 2003; Zenebe et al 2003). The aim of the current experiments was to evaluate the effect of Provinol on allergen-induced hyperreactivity of the airways. We investigated the effect of these polyphenols on tracheal smooth muscle reactivity in guinea pigs with allergen-induced hyperreactivity of the airways, and investigated the role of NO in this bronchodilatory effect.

Materials and Methods

Materials

Provinol (dry powder of red wine polyphenolic compounds) was provided by D. Ageron (Société Francaise de Distillerie, Vallont Pont d′Arc, France). The composition of Provinol was (in $mg\,g^{-1}$ dry powder): proanthocyanins 480, total anthocyanins 61, free anthocyanins 19, catechin 38, hydroxycinnamic acid 18, flavonols 14. Ovalbumin (egg albumin grade III), histamine hydrochloride, acetylcholine and other chemicals were purchased from Sigma Aldrich (Taufkirchen, Germany).

Sensitization of guinea pigs

Trik guinea pigs (200–250 g) of both sexes were actively sensitized by three injections of ovalbumin (in 1mL saline): 5 mg i.p. plus 5 mg s.c. on day 1, followed by 5 mg i.p. on day 4. The guinea pigs were used for experiments 14 days after the first dose of ovalbumin (Kreutner et al 1989). There were 12 animals in each experimental group. The experimental study was approved by the Ethical Committee of Jessenius Faculty of Medicine.

In vitro tracheal muscle contraction

The reactivity of guinea-pig tracheal smooth muscle was estimated in-vitro 14 days after the first injection of ovalbumin. Tracheal strips were placed in 20 mL organ baths containing Krebs–Henseleit buffer of the following composition (in glass-distilled water): NaCl, 110 μM; KCl, 4.8 μM; $CaCl_2$, 2.35 μM; $MgSO_4$, 1.2 μM; $KHPO_4$, 1.2 μM; $NaHCO_3$, 25 μM. Organ baths were maintained at 36.5 ± 0.5°C and were aerated continuously with 95%/5% oxygen/carbon dioxide to maintain pH at 7.5 ± 0.1. The tissue strips were initially set to 4 g tension (30 min loading phase). After this period, the tension in each tissue segment was readjusted to a baseline of 2 g (30 min adaptation phase). During these periods, the tissue was washed at 15 min intervals (Strapková and Nosál'ová 2002; Hudec et al 2003).

The amplitude of contraction (mN) of the tracheal smooth muscle in response to increasing doses of histamine (10^{-8}–$10^{-3}\,mol\,L^{-1}$), acetylcholine (10^{-8}–$10^{-3}\,mol\,L^{-1}$) and ovalbumin (10^{-5}–$10^{-3}\,g\,mL^{-1}$) was used as a parameter of tracheal smooth muscle reactivity (Bundschuh et al 2001).

Acute effect of Provinol

Isolated tracheal strips from the ovalbumin-sensitized guinea pigs were set up in the organ bath as described above and pretreated for 30 min with Provinol ($10^{-4}\,mg\,mL^{-1}$) alone or in combination with N^{ω}-nitro-L-arginine methyl ester (L-NAME; $10^{-6}\,mol\,L^{-1}$) (Karol 1994). This dose of Provinol was estimated from previous experiments in which $10^{-4}\,mg\,mL^{-1}$ Provinol induced maximal activation of c-NOS and relaxation of femoral arteries (Zenebe et al 2003). Control tracheal strips were prepared from ovalbumin-sensitized guinea pigs but did not receive any pretreatment.

Long-term effect of Provinol

The long-term effect of Provinol on tracheal smooth muscle reactivity was measured in tracheal tissue from three groups of ovalbumin-sensitized guinea pigs. One group was treated with Provinol, 20 mg per day administered orally for 14 days; the second group received this dose of Provinol plus L-NAME,

40 mg per day orally. The control group were ovalbumin-sensitized guinea pigs and were not given Provinol or L-NAME. This dose of Provinol corresponded to 10 times the concentration that produced maximal relaxation of endothelial smooth muscle after absorption from the digestive tract (Diebolt et al 2001). After a period of 14 days, the tracheal smooth muscle reactivity to bronchoconstrictors (histamine and acetylcholine) and to allergen (ovalbumin) was investigated in-vitro as described above.

Data analysis

Statistical analysis was performed using one-way analysis of variance. Differences were considered statistically significant when the P value was below 0.05. All results are expressed as mean ± s.e.m.

Results

Increased reactivity of airway smooth muscle is one feature of allergen-induced hyperreactivity of airways in an animal model. The degree of hyperreactivity in in-vitro conditions is assessed by an increased amplitude of contraction in response to bronchoconstrictors (Karol 1994). We used tracheal strips prepared from guinea pigs 14 days after sensitization with ovalbumin. Consistent with our previous study, the amplitude of tracheal smooth muscle contraction was increased by histamine (10 nM–1 mM) and acetylcholine (10 nM–1 mM) in a concentration-dependent manner (Varcholová et al 2003).

This animal model of allergen-induced hyperreactivity of the airways was used to evaluate the bronchodilatory activity of Provinol after acute pre-treatment in-vitro as well as after 14 days' oral administration. An additional step of the experiment was to confirm the role of NO in the bronchodilatory effect of Provinol.

Acute effect of Provinol

The stripped tracheal smooth muscles from the control, Provinol and Provinol plus L-NAME groups were placed in the organ bath 30 min before the bronchoconstrictor was added. The amplitude of contraction caused by histamine was significantly lower in the Provinol group than in the control group but was slightly higher than the control group in the Provinol plus L-NAME group (Figure 1).

Compared with controls, 30 min' pre-treatment with Provinol significantly inhibited the contraction induced by acetylcholine, but this inhibition was reduced by L-NAME (Figure 2).

During antigen-evoked tracheal smooth muscle contraction, 30 min' incubation with Provinol caused an inhibition of the tonic contraction. The effect of Provinol was inhibited by L-NAME, mainly at low doses of ovalbumin (Figure 3).

Long-term effect of Provinol

The long-term effect of Provinol, alone and in combination with L-NAME, on tracheal smooth muscle reactivity in ovalbumin-sensitized guinea pigs was evaluated after 14 days' administration. After this period, tracheal smooth muscle strips were contracted with cumulative doses of specific bronchoconstrictors (histamine and acetylcholine) as well as non-specific spasmogen (ovalbumin). Fourteen days' pre-treatment with Provinol inhibited the contraction induced by histamine, but this effect of Provinol was not affected by the non-selective NOS inhibitor L-NAME (Figure 4). In the case of acetylcholine-induced contraction, 14 days' administration of Provinol decreased the amplitude of tracheal smooth muscle contraction, and this effect was partially diminished by L-NAME, mainly at lower doses of acetylcholine (Figure 5).

In comparison with the acute experiment, long-term administration of Provinol inhibited the allergen (ovalbumin)-induced contraction of tracheal smooth muscle; the combination of Provinol plus L-NAME also blocked the bronchodilatory effect of Provinol but less so than Provinol alone (Figure 6).

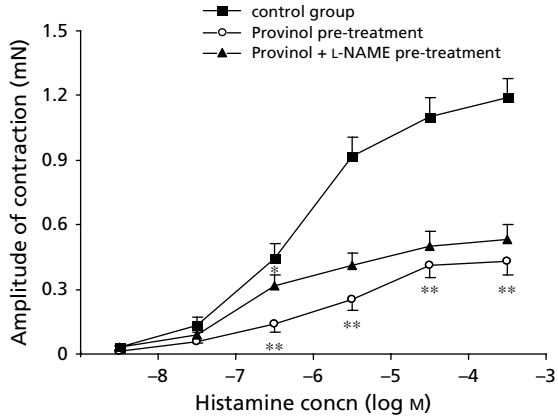

Figure 1 Amplitude of tracheal smooth muscle contraction in response to histamine after 30 min' pre-treatment with Provinol, alone or in combination with L-NAME, in guinea pigs sensitized with ovalbumin. Data are mean ± s.e.m.; n = 12 for each group. *$P < 0.05$; **$P < 0.01$.

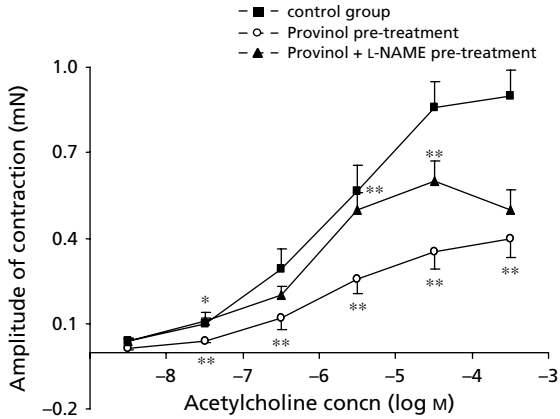

Figure 2 Amplitude of tracheal smooth muscle contraction in response to acetylcholine after 30 min' pre-treatment with Provinol, alone or in combination with L-NAME, in guinea pigs sensitized with ovalbumin. Data are mean ± s.e.m.; n = 12 for each group. *$P < 0.05$; **$P < 0.01$.

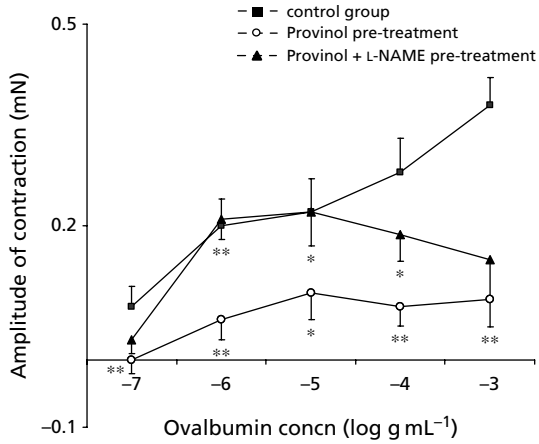

Figure 3 Amplitude of tracheal smooth muscle contraction in response to doses of allergen (ovalbumin) after 30 min' pre-treatment with Provinol, alone or in combination with L-NAME, in guinea pigs sensitized with ovalbumin. Data are mean ± s.e.m.; n = 12 for each group. *$P < 0.05$; **$P < 0.01$.

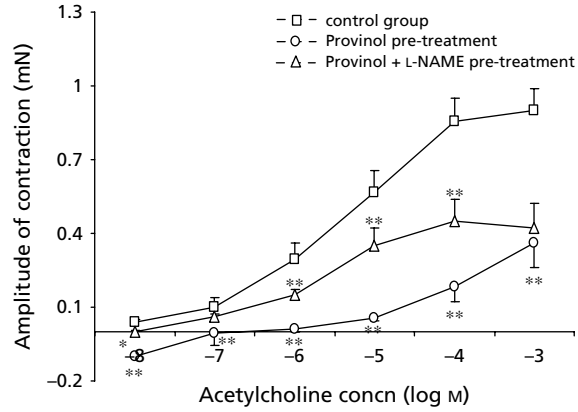

Figure 5 Amplitude of tracheal smooth muscle contraction in response to increasing doses of acetylcholine after 14 days' administration of Provinol, alone or in combination with L-NAME, in guinea pigs sensitized with ovalbumin. Data are mean ± s.e.m.; n = 12 for each group. *$P < 0.05$; **$P < 0.01$.

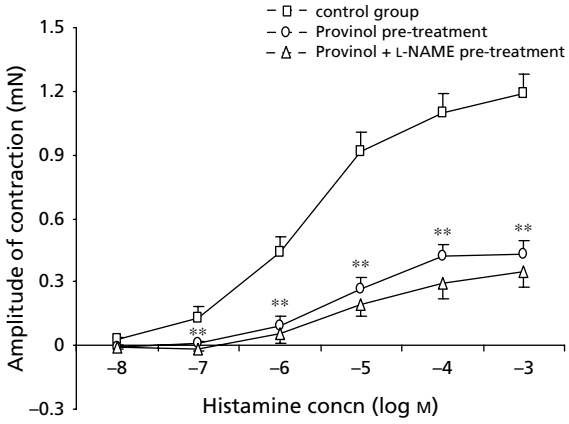

Figure 4 Amplitude of tracheal smooth muscle contraction in response to increasing doses of histamine after 14 days' administration of Provinol, alone or in combination with L-NAME, in guinea pigs sensitized with ovalbumin. Data are mean ± s.e.m.; n = 12 for each group. *$P < 0.05$; **$P < 0.01$.

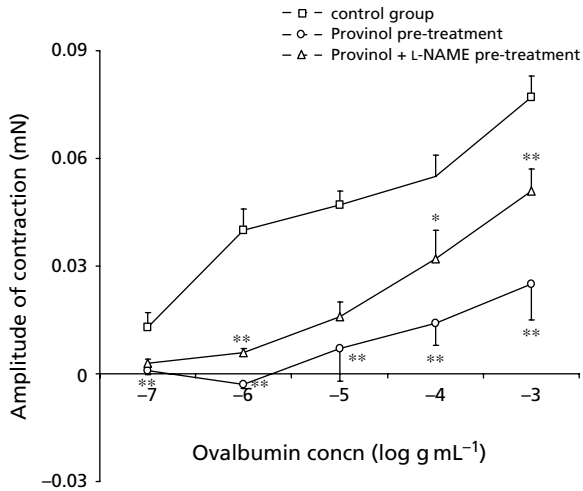

Figure 6 Amplitude of tracheal smooth muscle contraction to increasing doses of allergen (ovalbumin) after 14 days' administration of Provinol, alone or in combination with L-NAME, in guinea pigs sensitized with ovalbumin. Data are mean ± s.e.m.; n = 12 for each group. *$P < 0.05$; **$P < 0.01$.

Discussion

Provinol is a mixture of red wine polyphenolic compounds with a wide spectrum of biological activities. Provinol has been shown to have antithrombotic, cardioprotective, antihypertensive and anti-ischaemic activity and other positive effects (Zenebe and Pechanova 2002). The antihypertensive and cardioprotective effects of Provinol are mediated, at least in part, via NO-dependent pathways (Bernátová et al 2002).

In our experiments, Provinol inhibited tracheal smooth muscle contraction during allergen-induced hyperreactivity of the airways; this effect was partially mediated through NO metabolism.

Allergic asthma is characterized by allergen-induced early and late asthmatic reactions, airway inflammation and airway hyperreactivity to bronchoconstrictors (De Boer et al 1996). One of the important factors contributing to this pathological process is the imbalance in NO metabolism. Deficiency of constitutively formed NO, possibly resulting from epithelial damage due to inflammation, may contribute to allergen-induced airway hyperreactivity after an early asthmatic reaction (Nijkamp et al 1993). The inflammatory mediators decrease the function of cNOS, manifested by a lower bronchodilatory effect of NO. The neuronal NO-induced relaxation is impaired in allergic inflammation of the airways, indicating altered nNOS activity (Miura et al 1997). Stimulation of iNOS results in increased formation of inducible NO and asthmatic complications (Sanders 1999).

According to some hypotheses about the control of NO levels, physiological concentrations of NO or other substances able to stimulate cNOS under pathological conditions can suppress the activation of NF-κB, which thus contributes

to limiting processes such as immune response and inflammation (Marshall and Stamler 1999). Provinol at a concentration of 10^{-4} mg mL^{-1} in-vitro activated cNOS activity in the cardiovascular area (Zenebe 2003; Zenebe et al 2003).

In our experiments, incubation of tracheal smooth muscle strips with Provinol resulted in a decrease in the amplitude of contraction stimulated by bronchoconstrictors (histamine and acetylcholine). The non-selective NOS inhibitor L-NAME partially abolished the effect of Provinol on acetylcholine-induced, but not histamine-induced, bronchoconstriction. The inhibition of NOS by L-NAME in the airways interferes with the inhibitory non-adrenergic non-cholinergic neural bronchodilator response mediated by NO. In guinea-pig trachea, endogenous NO modulates cholinergic neural contractile responses (Ward et al 1993). Similar changes were observed after 14 days' administration of Provinol.

In the case of non-specific spasmogen-evoked contraction, both short- and long-term treatment with Provinol potently attenuated the ovalbumin-induced contraction of guinea-pig tracheal strips. The potency of Provinol in an allergen-driven pathological situation might be attributed to the inhibition of mast cell degranulation rather than to direct effects on the receptors responsible for bronchoconstriction (Underwood et al 1993). L-NAME antagonized the ovalbumin-induced contraction of tracheal smooth muscle strips prepared from sensitized guinea pigs after acute administration of Provinol, and with a lower potency after 14 days' administration. These results could indicate a decline in NO participation and the contribution of another mechanism in the longer-term effects of Provinol.

In the respiratory tract, polyphenols have many positive effects, including anti-inflammatory, anti-allergic and antioxidant properties. They are scavengers of NO and oxygen radicals (Van Acker et al 1995), and inhibit histamine release, cysteinyl leukotriene biosynthesis (Thomet et al 2002) and cytokine production (Sanbongi et al 2004). In our experiments, Provinol decreased the amplitude of tracheal smooth muscle contraction caused by bronchoconstrictors (histamine and acetylcholine) and to allergen during ovalbumin-induced hyperreactivity of the airways.

Provinol is a mixture of different polyphenolic compounds – flavonols (such as quercetin), flavans (such as catechins) and anthocyanins – and it is not certain which of these phenolic components are responsible for its activity. Another polyphenol from red wine, resveratrol, can downregulate the transcription factor NF-κB and thus influence NO metabolism (Holmes-McNary and Baldwin 2000).

Our results demonstrate that red wine polyphenolic compounds had a protective effect on the tracheal smooth muscle reactivity in response to bronchoconstrictor mediators and allergens in this model of ovalbumin-induced allergic asthma. It can be assumed that the mechanism of bronchodilatory effect of Provinol is probably mediated at least partly through the metabolism of NO.

Conclusion

The results of our experiments confirmed the ability of Provinol to decrease the amplitude of tracheal smooth muscle contraction in response to bronchoconstrictors (histamine and acetylcholine) and to allergen during allergen-induced hyperreactivity of the airways, and that the mechanism involved NO, at least in part.

The results indicate that the red wine polyphenolic compounds may become a supporting therapeutic option against airway hyperresponsiveness in patients with asthma.

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