

ORIGINAL ARTICLE

Effects of oral alpha-tocopherol on lung response in rat model of allergic asthma

JANA SUCHANKOVA,¹ MARIE VOPRSALOVA,¹ MARTINA KOTTOVA,¹ VLADIMIR SEMECKY² AND PETER VISNOVSKY¹

Departments of ¹Pharmacology and Toxicology, and ²Biological and Medical Sciences, Faculty of Pharmacy, Charles University, Prague, Czech Republic

Effects of oral alpha-tocopherol on lung response in rat model of allergic asthma

SUCHANKOVA J, VOPRSALOVA M, KOTTOVA M, SEMECKY V, VISNOVSKY P. *Respirology* 2006; **11**: 414–421

Objective and background: Asthma is a chronic inflammatory disease in which an oxidant/anti-oxidant imbalance plays an important role. D-alpha-tocopherol (biologically the most active form of vitamin E) has redox properties and by scavenging the free radicals can act as an antioxidant. The aim of this study was to examine the effects of orally administered alpha-tocopherol in a rat model of allergic asthma.

Methodology: Actively sensitized rats (OA) were treated with alpha-tocopherol (400 mg/kg/day for 10 days) or vehicle; 1 h after the last dose, they were challenged with antigen aerosol. The antigen-induced airway hyperresponsiveness to direct bronchoconstrictor (serotonin), the inflammatory cell infiltrate and histological changes were determined 1 or 24 h after the antigen challenge.

Results: Alpha-tocopherol pretreatment was not significantly effective at reducing the studied parameters when compared with controls, even though there was a tendency to a reduction in bronchial responsiveness and in eosinophil and neutrophil infiltration.

Conclusion: Alpha-tocopherol when administered in the chosen study design in an animal model of asthma had no major effect on airway inflammation. The effect of antioxidants deserves further evaluation.

Key words: alpha-tocopherol, antioxidant, asthma, ovalbumin, rat.

INTRODUCTION

Oxidative stress participates in the pathophysiology of a number of pulmonary diseases, including asthma. Healthy subjects compensate for the toxicity of free radicals and reactive oxygen species by means of endogenous defence systems (antioxidants and enzymes). In asthmatics, oxidant/antioxidant imbalances cause an oxidative stress that elicits a broad spectrum of pathophysiological effects. Although the oxidant/antioxidant imbalance is neither unique nor the key feature in the pathogenesis of asthma, its

role is very important and contributes to disease progression.^{1–4}

Naturally occurring vitamin E is a mixture of about eight tocopherols. Biologically the most active form is D-alpha-tocopherol, which represents about 90% of the tocopherols in animal tissue. Tocopherols can act as antioxidants. By scavenging free radicals, they mediate protection of important biological structures from oxidative damage. Tocopherol is regenerated by means of interaction with other antioxidant compounds, such as ascorbic acid.⁵

There exist many clinical reports documenting changes in antioxidant concentrations in asthmatics. Antioxidant vitamins are decreased in the sera of asthmatic children even during asymptomatic periods.⁶ The plasma and whole blood levels of alpha-tocopherol are decreased in asthmatics when compared with healthy volunteers.^{7,8} Furthermore, the lung-lining-fluid concentrations of vitamin E and vitamin C are low in patients with mild asthma even though blood levels are normal or increased.⁹ All

Correspondence: Jana Suchankova, Faculty of Pharmacy, Heyrovskeho 1203, Hradec Kralove, CZ-50005, Czech Republic. Email: jana.suchankova@faf.cuni.cz

Received 24 May 2005; invited to revise 28 October 2005; revised 28 January 2006; accepted 2 February 2006 (Associate Editor: Toshihiro Nukiwa).

these findings together with the presence of increased amount of oxidized glutathione in their airways indicate that patients with asthma are exposed to an increased oxidative stress.⁹

So far, very few experimental studies of asthma dealing with the effects of vitamin E have been reported. It has been recently shown that alpha-tocopherol pretreatment of ovalbumin-sensitized guinea pigs significantly reduces the contractility of tracheal preparation,¹⁰ and in another experiment, a weak protective effect on ovalbumin-induced bronchospasm in allergic guinea pigs has been observed.¹¹ Although antioxidant effects of vitamin E have been documented, its underlying mechanism is not fully understood.¹

As excess reactive oxygen species production and insufficient endogenous antioxidant defence mechanisms is a feature of the pathogenesis of asthma, we hypothesized that administration of antioxidants may be beneficial. Therefore the objective of this research was to study the effects of orally administered alpha-tocopherol on antigen-induced airway hyperresponsiveness to the direct bronchoconstrictor serotonin, the infiltration of inflammatory cells (mainly eosinophils and neutrophils) and on airway histological changes in a rat model of allergic asthma.

METHODS

All experiments were carried out with the approval of the Ethics Committee of the Faculty of Pharmacy, Charles University. The experimental protocol complied with the regulations established for use of laboratory animals by the Czech Government and the European Community.

Study design

Actively sensitized rats (OA) were treated with the study drug or vehicle for 10 days, and 1 h after the last dose, were challenged with antigen aerosol. Bronchial responsiveness to 5-hydroxytryptamine (5-HT) was determined 1 or 24 h after the antigen challenge. The bronchoalveolar lavage fluid (BALF) was obtained and analysed immediately after the measurement of lung responsiveness and the lungs were removed for histological examination.

Experimental groups

- Treated group: ovalbumin-sensitized animals treated with alpha-tocopherol and challenged with antigen aerosol ($n = 9$)
- Positive control group: ovalbumin-sensitized animals treated with a drug vehicle and challenged with antigen aerosol ($n = 9$)
- Negative control group: ovalbumin-sensitized animals treated with a drug vehicle and challenged with saline aerosol ($n = 10$)

Animals

Male Wistar rats with a weight of 240–260 g supplied by BioTest (Konarovice, Czech Republic) were used for this study. Animals were divided at random into experimental groups as indicated below and housed five to a cage under standard laboratory conditions.

Drugs

The following drugs were used: D-alpha-tocopherol (M.W. = 430.7 Da, 1000 IU/g in soya oil), soya oil (vehicle), ovalbumin (min 98%), aluminium hydroxide (M.W. = 78 Da), succinylcholine chloride (M.W. = 397.3 Da), DL-propranolol hydrochloride (M.W. = 295.8 Da), 5-HT hydrochloride (M.W. = 212.7 Da) and sodium pentobarbital (M.W. = 248.3 Da). Drugs were obtained from the Sigma and Fluka Company (Sigma Aldrich s.r.o., Prague, Czech Republic).

Sensitization

Animals were actively sensitized as previously described by Elwood and this procedure has previously been shown to result in the development of IgE antibody.¹² This model of allergic inflammation has been applied to various animal species (rat, guinea pig, mouse) and has been used previously for the studies of antioxidants. The induced changes in the lung oxidative status have been reported in the literature.^{13–16} Ovalbumin OA (1 mg) and aluminium hydroxide $\text{Al}(\text{OH})_3$ (100 mg) were suspended in 0.9% w/v saline (1 mL) and intraperitoneally injected into each rat. Injection was repeated for three consecutive days. Sensitized animals were used in experiments from day 21 to day 30 after the initial intraperitoneal injection.

Aerosol—inhale system

The inhalation system consisted of a transparent plastic chamber (approximate volume of 4 L) that was connected to the output of a jet HSE nebulizer (Hugo Sachs Elektronik, Harvard Apparatus GmbH, Grunstrasse 1, D 79232 March—Hugstetten, Germany). The particles of generated aerosol were less than 10 μm in size with 60% of the particles being less than 2.5 μm (the size was tested and declared by the HSE). The nebulizer output was approximately 18–20 mL/h. For allergen challenge, each rat was placed in the chamber that was then filled with aerosol.

Allergen challenge

Within a time interval of 30 min, the animals were exposed to ovalbumin aerosol (100 mg in 2 mL of saline). Sham group (negative controls) was challenged with saline.

Drug treatment

Animals were treated with alpha-tocopherol at 400 mg/kg/day or vehicle for 10 days and the last dose was administered 1 h before the ovalbumin challenge. The oral route (by means of gavage) was selected as it best represents the clinical setting. The dose administered and the chosen study design were derived on the basis of previous studies on the antioxidative properties of alpha-tocopherol on rats. Repeated administration was selected because we assumed that a more pronounced effect was likely to be observed.^{17–21}

Animal preparation

At 1 or 24 h after the antigen challenge, the animals were anaesthetized with pentobarbital (45 mg/kg i.p.). The surgical anaesthesia was maintained during the whole experiment (for approx. 45 min) without the need for the supplementary pentobarbital administration. A heparinized catheter was inserted into the right jugular vein for i.v. drug administration. After the tracheostomy, the trachea was cannulated and connected to a Hugo Sachs ventilator (90 breaths/min, 10 mL per kilogram of body weight). Spontaneous respiration was suppressed by an i.v. succinylcholine injection (2 mg/kg) and the animals were mechanically ventilated with room air.

Measurement of bronchial responsiveness

The classical method of Konzett-Roessler was applied. The overflow (i.e. air volume not taken up by the lungs) was measured by the Fleisch pneumotachometer (Hugo Sachs Elektronik, Harvard Apparatus, March-Hugstetten, Germany) and recorded by a differential pressure transducer as airflow in mL/s. The tidal volume was calculated after each respiratory cycle by use of HSE Pulmodyn Software (Hugo Sachs Elektronik). The increase in overflow indicated the bronchoconstrictive response. Five minutes before the first 5-HT dose, the animals were pretreated with propranolol (1 mg/kg i.v.) in order to produce a large bronchoconstrictor response. Bronchial responsiveness was determined from a dose-response curve to 5-HT (5, 10, 25 and 50 µg/kg i.v.) 1 or 24 h after the challenge. 5-HT was administered with a 3-min interval between doses.

BALF acquisition and analysis

After the measurement of lung responsiveness, the rats were disconnected from the ventilator and sacrificed with the pentobarbital overdose. The lungs were lavaged with two aliquots, each having 6 mL of saline, and the recovered BALF was pooled. The cell suspension was concentrated by centrifugation (1200 r.p.m., 20 min, 4°C), the cell pellet was resuspended in 1 mL of saline and total and differential cell counts were made.

Histopathological examination

Whole rat lungs including the trachea were removed for histological examination and immediately immersed in Bouin's fixative solution. Forty-eight hours later, the tissue samples were embedded in paraffin. Some paraffin sections of rat lung (thickness of 5–7 µm) were stained with haematoxylin and eosin as appropriate with combination Alcian blue-haematoxylin-eosin for staining of acid and neutral mucin or Masson's trichrom staining. Stained slides were studied by light-microscopy using computer image analysis and photodocumentation was provided with the Nikon Eclipse E200 microscope, the digital firewire camera PixeLINK PL-A642 (Vitana Corp., Ottawa, Canada) and with image analysis software LUCIA (Laboratory Imaging, Prague, Czech Republic).

Data analysis

Data are presented as mean $\pm$ SEM of n experiments. A statistical analysis of the results was carried out by means of Student's t -test and P -value of <0.05 was considered significant.

RESULTS

Bronchial responsiveness

One hour after the allergen challenge, i.v. serotonin administration (5–50 µg/kg) induced a dose-related bronchoconstrictor response in all rats. The bronchospasm in sensitized and OA-challenged animals was not significantly augmented when compared with sham group, and no differences in airway response were found after the alpha-tocopherol pretreatment (data not shown).

Within a time interval of 24 h after antigen challenge, i.v. cumulative administration of serotonin induced a dose-dependent and significant increase in overflow volume when the OA-immunized and -challenged animals were compared with the saline-challenged controls (after the last serotonin dose the increase in overflow volume was $753 \pm 34\%$ $n=9$ and $396 \pm 34\%$ $n=10$, respectively). In tocopherol-pretreated animals, no significant changes were found. A tendency to an antihyperresponsiveness effect was observed when compared with the non-pretreated antigen-challenged group ($558 \pm 41\%$; $n=9$) (Fig. 1).

BALF analysis

BAL was carried out 1 or 24 h after saline or ovalbumin exposure. One hour after the antigen challenge, no differences in the total cell count were found among the groups. The total number of cells in the BALF obtained from sham and control groups and from treated animals was 0.24 ± 0.02 $n=9$; 0.33 ± 0.05 $n=8$ and 0.30 ± 0.03 $n=9$ ($10^6/\text{mL}$). Similarly, no

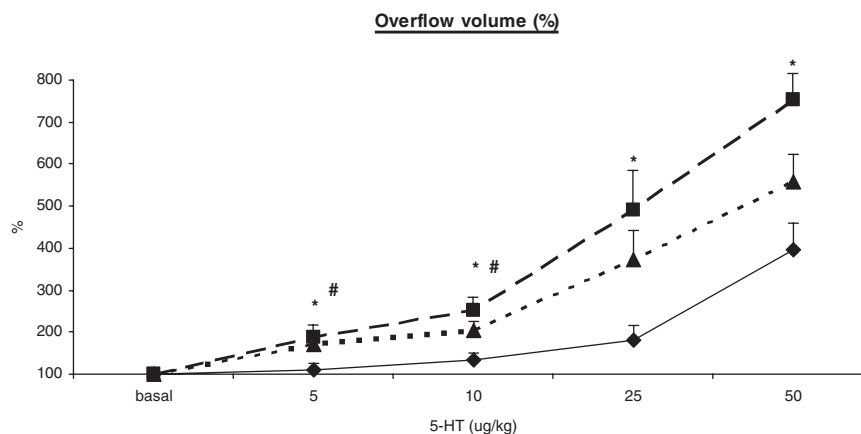


Figure 1 Dose-response curves for i.v. 5-hydroxytryptamine (5-HT) were obtained in Wistar rats exposed 24 h earlier to aerosol saline (negative control) or antigen (OA 1%, 15 min). The antigen-challenged animals received by means of gavage a drug vehicle (positive control) or alpha-tocopherol (400 mg/kg/day for 10 days, the last dose 1 h prechallenge) as indicated. The increase in overflow is presented as percentage change from baseline values. The airway hyperresponsiveness to 5-HT observed in antigen-challenged sensitized rats tended to decrease after alpha-tocopherol. The points are mean $\pm$ SEM of 10 (negative control), 9 (positive control) and 9 (alpha-tocopherol) animals; * $P < 0.05$ from negative control; # $P < 0.05$ from positive control. (—◆—) Sensitized and saline-challenged group receiving the drug vehicle, 24 h post challenge; (—■—) sensitized and OA-challenged group receiving the drug vehicle, 24 h post challenge; and (---▲---) sensitized and OA-challenged group receiving alpha-tocopherol, 24 h post challenge.

changes were found in the differential cell count (data not shown).

Twenty-four hours after antigen exposure, OA-challenged animals showed a significantly higher total number of cells in BALF when compared with saline-challenged controls (0.97 ± 0.09 , $n = 9$ and 0.24 ± 0.02 , $n = 10$ ($10^6/\text{mL}$)). The increase in total cell number was not significantly changed by tocopherol, although there was a tendency for it to be lower (0.60 ± 0.08 , $n = 9$ ($10^6/\text{mL}$)). The differential cell count showed an increase in eosinophils (0.133 ± 0.028 , $n = 9$ and 0.005 ± 0.001 , $n = 10$ ($10^6/\text{mL}$)) and significant increase in neutrophils (0.342 ± 0.046 , $n = 9$ and 0.007 ± 0.001 , $n = 10$ ($10^6/\text{mL}$)) in the recovered BALF of antigen-challenged rats compared with the saline exposed group. The increased percentage of eosinophils and neutrophils tended to diminish in tocopherol treated rats (0.039 ± 0.011 , $n = 9$ and 0.122 ± 0.032 , $n = 9$) but the difference was not significant (Figs 2,3).

Histopathological examination

The histopathological examination of both OA-challenged animals and alpha-tocopherol-treated animals showed inflammatory changes when compared with saline-challenged controls. In examined lung sections, a distinct inflammatory infiltrate was seen in peribronchial and perivascular areas originating centrally and extending along the vessels and bronchi into the marginal lung parenchyma. Occasionally there was a mild thickening and constriction of the walls in a small number of bronchi and bronchioles and likewise hypersecretion of mucus by goblet cells. The peribronchial and perivascular inflammatory infiltrate predominantly consisted of eosinophils and mast cells, admixed with lympho-

cytes, macrophages and rarely histiocyte-like cells. The infiltrate expanded into the lung interstitium and was associated with local oedema. In alpha-tocopherol-premedicated animals, no differences in quantitative and qualitative parameters were found when compared with OA-challenged controls. In the interstitial area, there was a similar peribronchial and perivascular inflammation with predominant eosinophils and lymphocytes. Hypertrophy of the smooth muscle cell layer was noted in the alpha-tocopherol-pretreated rats after antigen exposure but this was comparable to the findings in OA-challenged control group (Fig. 4).

DISCUSSION

Asthma is a chronic inflammatory disease characterized by airway obstruction, lung hyperresponsiveness, enhanced airways vascular permeability accompanied by oedema, enhanced mucus secretion, and inflammatory cell infiltration.^{22–24} Oxidative stress significantly participates in its pathogenesis, with inflammatory cells having a fundamental contributing role as they release increased amounts of reactive oxygen species derivatives (ROS).⁴ Although all inflammatory cells participate in asthma pathogenesis, the airway eosinophilia appears to be of central importance.^{25–27} Other cells, such as neutrophils also contribute to this process, even though their role seems less important. The migration of inflammatory cells depends on a number of factors, including the expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1).^{27,28} The ICAM-1 and VCAM-1 expressions are nuclear factor NF- κ B-dependent,^{29,30} with this transcription factor being sensitive to oxidants.³¹

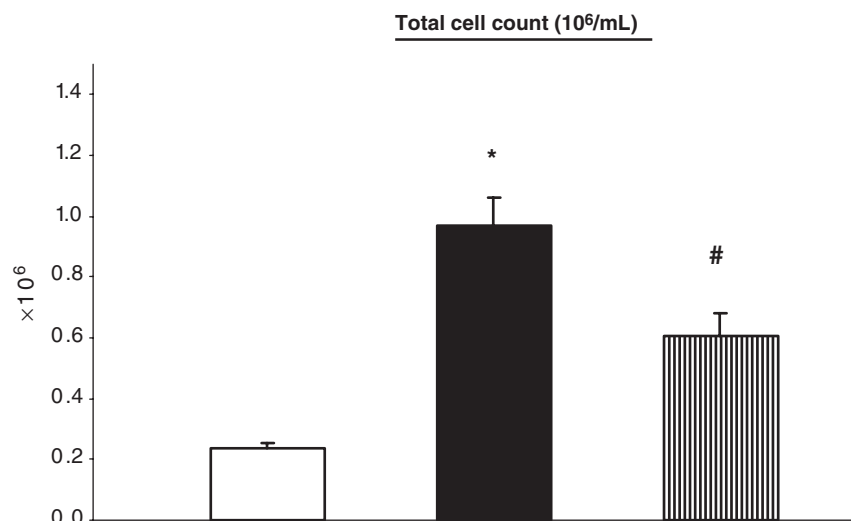


Figure 2 Total cell numbers in bronchoalveolar lavage fluid are shown for sensitized Wistar rats exposed 24 h earlier to aerosol saline (negative control) or antigen (OA 1%, 15 min). The antigen-challenged animals received by means of gavage a drug vehicle (positive control) or alpha-tocopherol (400 mg/kg/day for 10 days, the last dose 1 h prechallenge) as indicated. Alpha-tocopherol administration slightly reduced the augmented total cell number in bronchoalveolar lavage fluid of antigen-challenged sensitized rats. The columns are mean $\pm$ SEM of 10 (negative control), 9 (positive control) and 9 (alpha-tocopherol) animals; * P < 0.05 from negative control; # P < 0.05 from positive control. (□) Sensitized and saline-challenged group receiving the drug vehicle, 24 h post challenge; (■) sensitized and OA-challenged group receiving the drug vehicle, 24 h post challenge; and (▨) sensitized and OA-challenged group receiving alpha-tocopherol, 24 h post challenge.

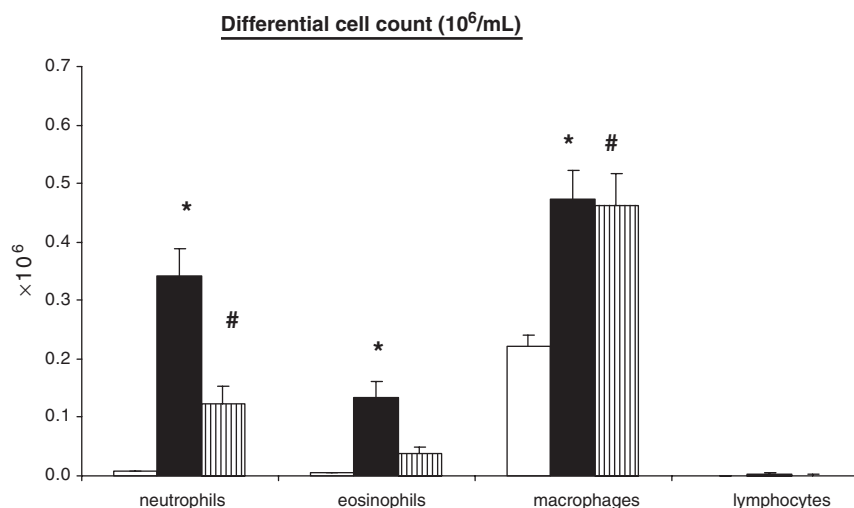
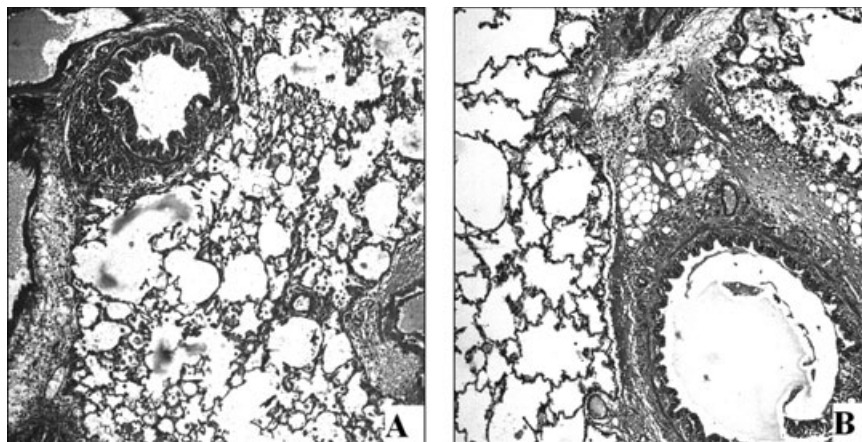


Figure 3 Eosinophil, neutrophil, lymphocyte and macrophage numbers in bronchoalveolar lavage fluid are shown for sensitized Wistar rats exposed 24 h earlier to aerosol saline (negative control) or antigen (OA 1%, 15 min). The antigen-challenged animals received by means of gavage a drug vehicle (positive control) or alpha-tocopherol (400 mg/kg/day for 10 days, the last dose 1 h prechallenge) as indicated. After alpha-tocopherol administration, a significant reduction in the increased number of neutrophils and a tendency for eosinophil numbers to decrease were observed in antigen-challenged sensitized rats. The columns are mean $\pm$ SEM of 10 (negative control), 9 (positive control) and 9 (alpha-tocopherol) animals; * P < 0.05 from negative control; # P < 0.05 from positive control. (□) Sensitized and saline-challenged group receiving the drug vehicle, 24 h post challenge; (■) sensitized and OA-challenged group receiving the drug vehicle, 24 h post challenge; and (▨) sensitized and OA-challenged group receiving alpha-tocopherol, 24 h post challenge.

Accordingly, antioxidant treatment has been demonstrated to inhibit the expression of both adhesion molecules through NF- κ B-dependent and even NF- κ B-independent pathways.³² Moreover, a reducing

effect on eosinophilia has been reported after an oral administration of some antioxidants.^{13,33,34} A relationship between oxidative stress and neutrophil traffic in the lungs has also been observed.^{4,35,36}

Figure 4 Representative histopathological findings of alpha-tocopherol-treated animals (A) and OA-challenged animals (B) are shown (haematoxylin and eosin staining ($\times 100$) of rat pulmonary tissue). The peribronchial and perivascular inflammation (predominantly consisted of eosinophils and lymphocytes) and the hypertrophy of the smooth muscle were present in both groups, and no differences were found.



We hypothesized that the administration of alpha-tocopherol, an antioxidant vitamin, may reduce the antigen-induced airway hyperresponsiveness and the inflammatory cells infiltrate in a rat model of allergic asthma. Contrary to our hypothesis, alpha-tocopherol pretreatment was not significantly effective at reducing the studied parameters when compared with controls. This observation can be explained in three ways.

First, our hypothesis was not correct and despite its antioxidative properties, alpha-tocopherol, regardless of the dose and administration schedule, does not affect the increased bronchial responsiveness and pulmonary inflammation induced by allergen challenge. As other antioxidants have been effective in similar experimental models,^{13,33,34} a relationship between the oxidant/antioxidant ratio and the response after allergen challenge is very likely to exist. The organism defence against oxidants is secured by a complex antioxidant system, with its components playing different roles. Role in the system determines the importance of each antioxidant under particular oxidative stress conditions. Hence, the way exogenous antioxidants are integrated into the physiological antioxidative defence systems determines their effectiveness. For example, the thiol compound N-acetylcysteine (NAC) acts directly as a free radical scavenger, and also as a precursor of reduced glutathione (a crucial component of the antioxidant defence). Thus, NAC pretreatment in an experimental model of asthma has significant effects on antigen-induced airway hyperresponsiveness, lipid peroxidation and expression of some enzymes and factors.^{13,37} As mentioned above, alpha-tocopherol is an antioxidant vitamin with redox properties that can scavenge free radicals and its regeneration involves interaction with other antioxidants. The zero effect of alpha-tocopherol might be explained as being a result of a less important role of alpha-tocopherol in responding to ovalbumin challenge in actively sensitized rats OA. Possibly, the amount of alpha-tocopherol is not crucial for efficiency of the antioxidant system and it is not essential for the formation and/or regeneration of the main antioxidants (e.g. reduced glutathione) and/or their direct precursors either. Participation of fac-

tors other than oxidants and the complex character of asthma pathogenesis need also to be taken into account.

Second, the chosen asthma model may not be appropriate and does not enable the verification of our hypothesis. This possibility, however, is unlikely. We found significant differences in the bronchial reactivity of sensitized and sham experimental groups within the time interval of 24 h and histopathological examination showed inflammatory changes. These results are consistent with the generally accepted concept of a late reaction in sensitized and challenged rats^{13,33,34} and also confirm that the time interval of 1 h after antigen exposure probably is not sufficient for the development of measurable changes. Moreover, the experimental model of allergic asthma that was used has been widely employed in similar studies and the sensitization procedure and antigen challenge applied have been effective in those with similar study design. Rats together with guinea pigs are the most widely used species in both *in vivo* and *in vitro* experimental asthma investigations. The Wistar rats have been employed in experiments based on the ovalbumin sensitization,^{38–40} and in other lung-related studies as well.^{41,42} Nevertheless we do not exclude the possibility of obtaining more consistent results with, for example, Brown Norway rats, which can exhibit higher numbers of neutrophils and eosinophils.

Third, alpha-tocopherol might be able to alter the response to ovalbumin challenge in actively sensitized rats OA, but the doses used were inappropriate. We did not find any significant changes after alpha-tocopherol treatment, even though there was a tendency to reduce in bronchial responsiveness and eosinophil and neutrophil number. This might be because of the antioxidative properties of alpha-tocopherol.

This is the first description of the effects of vitamin E on the lung response in an experimental rat model of allergic asthma. Although our results are negative, they do not exclude the potential therapeutic value of antioxidants. The effects of antioxidants or their mixtures have not been investigated in detail, and deserve greater attention and further research.

ACKNOWLEDGEMENTS

The authors express their gratitude to Mrs Jitka Michlova and Mrs Blanka Slechtova for their expert technical assistance. The research described in this paper was supported by the Research Project CEZ13/98: 11600002 Grant.

REFERENCES

- 1 Stipek S, Borovansky J, Cejkova J *et al.* *Antioxidanty a Volne Radikaly Ve Zdravi a Nemoci*, 1st edn. Grada Publishing, Prague, 2000.
- 2 Fujisawa T. Role of oxygen radicals on bronchial asthma. *Curr. Drug Targets Inflamm. Allergy* 2005; **4**: 505–9.
- 3 Agacdiken A, Basyigit I, Ozden M *et al.* The effects of antioxidants on exercise-induced lipid peroxidation in patients with COPD. *Respirology* 2004; **9**: 38–42.
- 4 MacNee W. Oxidative stress and lung inflammation in airways disease. *Eur. J. Pharmacol.* 2001; **429**: 195–207.
- 5 Marcus R, Coulston AM. Fat-soluble vitamins. In: Hardman JG, Limbird LE, Gilman AG (eds). *The Pharmacological Basis of Therapeutics*, 10th edn. The McGraw–Hill Companies, Inc, New York, 2001; 1786–8.
- 6 Kalayci O, Besler T, Kilinc K, Sekerel BE, Saraclar Y. Serum levels of antioxidant vitamins (alpha tocopherol, beta carotene, and ascorbic acid) in children with bronchial asthma. *Turk. J. Pediatr.* 2000; **42**: 17–21.
- 7 Gazdik F, Gvozdzakova A, Horvathova M *et al.* Levels of coenzyme Q10 in asthmatics. *Bratisl. Lek. Listy* 2002; **103**: 353–6.
- 8 Gazdik F, Gvozdzakova A, Nadvornikova R *et al.* Decreased levels of coenzyme Q (10) in patients with bronchial asthma. *Allergy* 2002; **57**: 811–14.
- 9 Kelly FJ, Mudway I, Blomberg A, Frew A, Sandstrom T. Altered lung antioxidant status in patients with mild asthma. *Lancet* 1999; **354**: 482–3.
- 10 Kilic FS, Erol K. The effects of vitamin E in ovalbumin-sensitized guinea pigs. *Methods Find. Exp. Clin. Pharmacol.* 2003; **25**: 27–31.
- 11 Stetinova V, Grossmann V. Effects of known and potential antioxidants on animal models of pathological processes (diabetes, gastric lesions, allergic bronchospasm). *Exp. Toxicol. Pathol* 2000; **52**: 473–9.
- 12 Elwood W, Barnes PJ, Chung KF. Airway hyperresponsiveness is associated with inflammatory cell infiltration in allergic brown-Norway rats. *Int. Arch. Allergy Immunol.* 1992; **99**: 91–7.
- 13 Blesa S, Cortijo J, Mata M *et al.* Oral N-acetylcysteine attenuates the rat pulmonary inflammatory response to antigen. *Eur. Respir. J.* 2003; **21**: 394–400.
- 14 Shvedova AA, Kisin ER, Kagan VE, Karol MH. Increased lipid peroxidation and decreased antioxidants in lungs of guinea pigs following an allergic pulmonary response. *Toxicol. Appl. Pharmacol.* 1995; **132**: 72–81.
- 15 Nie X, Li Q, Cai G, Dai Y, Zhang J. The effect of N-acetylcysteine on Clara cells and Clara cell 16 kDa protein in a murine model of allergen-induced airway inflammation. *Respirology* 2005; **10**: 157–63.
- 16 Boskabady MH, Ziaei T. Effect of ascorbic acid on airway responsiveness in ovalbumin sensitized guinea pigs. *Respirology* 2003; **8**: 473–8.
- 17 MacDonald-Wicks LK, Garg ML. Vitamin E supplementation in the mitigation of carbon tetrachloride induced oxidative stress in rats. *J. Nutr. Biochem.* 2003; **14**: 211–18.
- 18 Helen A, Krishnakumar K, Vijayammal PL, Augusti KT. A comparative study of antioxidants S-allyl cysteine sulfoxide and vitamin E on the damages induced by nicotine in rats. *Pharmacology* 2003; **67**: 113–17.
- 19 Demiralay R, Gursan N, Erdem H. The effects of erdosteine, N-acetylcysteine, and vitamin E on nicotine-induced apoptosis of pulmonary cells. *Toxicology* 2006; **219**: 197–207.
- 20 Frenoux JM, Noiro B, Prost ED *et al.* Very high alpha-tocopherol diet diminishes oxidative stress and hypercoagulation in hypertensive rats but not in normotensive rats. *Med. Sci. Monit.* 2002; **8**: 401–7.
- 21 Zhang Y, Turunen M, Appelkvist EL. Restricted uptake of dietary coenzyme Q is in contrast to the unrestricted uptake of alpha-tocopherol into rat organs and cells. *J. Nutr.* 1996; **126**: 2089–97.
- 22 Ellis KM, Cannet C, Mazzoni L, Fozard JR. Airway hyperresponsiveness to bradykinin induced by allergen challenge in actively sensitised Brown Norway rats. *Naunyn Schmiedebergs Arch. Pharmacol.* 2004; **369**: 166–78.
- 23 Nagao K, Akabane H, Masuda T, Komai M, Tanaka H, Nagai H. Effect of MX-68 on airway inflammation and hyperresponsiveness in mice and guinea-pigs. *J. Pharm. Pharmacol.* 2004; **56**: 187–96.
- 24 Tanaka H, Komai M, Nagao K *et al.* Role of IL-5 and eosinophils in allergen-induced airway remodeling in mice. *Am. J. Respir. Cell. Mol. Biol.* 2004; **31**: 62–8.
- 25 Wood LG, Gibson PG, Garg ML. Biomarkers of lipid peroxidation, airway inflammation and asthma. *Eur. Respir. J.* 2003; **21**: 177–86.
- 26 Yamamoto K, Takanashi S, Hasegawa Y, Kanehira Y, Kaizuka M, Okumura K. Eotaxin level in induced sputum is increased in patients with bronchial asthma and in smokers. *Respiration* 2003; **70**: 600–5.
- 27 Adamko D, Lacy P, Moqbel R. Eosinophil function in allergic inflammation: from bone marrow to tissue response. *Curr. Allergy Asthma Rep.* 2004; **4**: 149–58.
- 28 Kaifi JT, Diaconu E, Pearlman E. Distinct roles for PECAM-1, ICAM-1, and VCAM-1 in recruitment of neutrophils and eosinophils to the cornea in ocular onchocerciasis (river blindness). *J. Immunol.* 2001; **166**: 6795–801.
- 29 Holden NS, Catley MC, Cambridge LM, Barnes PJ, Newton R. ICAM-1 expression is highly NF-kappaB-dependent in A549 cells. *Eur. J. Biochem.* 2004; **271**: 785–91.
- 30 Kim I, Moon SO, Kim SH, Kim HJ, Koh YS, Koh GY. Vascular endothelial growth factor expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin through nuclear factor-kappa B activation in endothelial cells. *J. Biol. Chem.* 2001; **276**: 7614–20.
- 31 Yang L, Cohn L, Zhang DH, Homer R, Ray A, Ray P. Essential role of nuclear factor kappaB in the induction of eosinophilia in allergic airway inflammation. *J. Exp. Med.* 1998; **188**: 1739–50.
- 32 Chang LY, Crapo JD. Inhibition of airway inflammation and hyperreactivity by an antioxidant mimetic. *Free Radic. Biol. Med.* 2002; **33**: 379–86.

- 33 Cortijo J, Blesa S, Martinez-Losa M *et al.* Effects of taurine on pulmonary responses to antigen in sensitized Brown-Norway rats. *Eur. J. Pharmacol.* 2001; **431**: 111–17.
- 34 Blesa S, Cortijo J, Martinez-Losa M *et al.* Effectiveness of oral N -acetylcysteine in a rat experimental model of asthma. *Pharmacol. Res.* 2002; **45**: 135–40.
- 35 Drost EM, Selby C, Lannan S, Lowe GD, MacNee W. Changes in neutrophil deformability following in vitro smoke exposure: mechanism and protection. *Am. J. Respir. Cell. Mol. Biol.* 1992; **6**: 287–95.
- 36 Lehr HA, Kress E, Menger MD *et al.* Cigarette smoke elicits leukocyte adhesion to endothelium in hamsters: inhibition by CuZn-SOD. *Free Radic. Biol. Med.* 1993; **14**: 573–81.
- 37 Cotgreave IA. N-acetylcysteine: pharmacological considerations and experimental and clinical applications. *Adv. Pharmacol.* 1997; **38**: 205–27.
- 38 Ferreira HH, Costa RA, Jacheta JM *et al.* Modulation of eosinophil migration from bone marrow to lungs of allergic rats by nitric oxide. *Biochem. Pharmacol.* 2004; **68**: 631–9.
- 39 Xu S, Xu Y, Zhang Z, Ni W, Chen S. Effect of nuclear factor-kappaB on airway remodeling in asthmatic rats. *J. Huazhong. Univ. Sci. Technol. Med. Sci.* 2004; **24**: 13–18.
- 40 Feitosa RF, Melciades GB, Assreuy AM, Rocha MF, Ribeiro RA, Lima AA. The pharmacological profile of ovalbumin-induced paw oedema in rats. *Mediators Inflamm.* 2002; **11**: 155–63.
- 41 Pauluhn J. Comparative analysis of pulmonary irritation by measurements of Penh and protein in bronchoalveolar lavage fluid in brown Norway rats and Wistar rats exposed to irritant aerosols. *Inhal. Toxicol.* 2004; **16**: 159–75.
- 42 Dye JA, Madden MC, Richards JH, Lehmann JR, Devlin RB, Costa DL. Ozone effects on airway responsiveness, lung injury, and inflammation. Comparative rat strain and *in vivo/in vitro* investigations. *Inhal. Toxicol.* 1999; **11**: 1015–40.