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EXPERIMENTAL STUDY

Acute bronchodilator effect of quercetin in experimental allergic asthma

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Abstract: *Objectives:* The aim of our study was to investigate the acute effect of quercetin on experimental allergic asthma after single-dose oral administration.
Background: Airway hyperresponsiveness is one of the main features of allergic asthma. None of quercetin experimental studies analysed the acute effect of this flavonol on the reactivity of airways both, *in vivo* and *in vitro* conditions.
Methods: Our experiment was realized 21 days after the sensitization of guinea pigs with ovalbumin suspension. Changes in the reactivity of airways were studied using the whole body plethysmography in order to compare changes of the specific airway conductance between groups with and without quercetin treatment. Also changes in the reactivity of the tracheal smooth muscle dipped into the organ bath with Krebs-Henseleit solution were measured as the reaction on cumulative doses of the bronchoconstrictor mediators histamine and acetylcholine. Quercetin was added into the solution 30 minutes before the chemical mediators. The amplitude of tracheal smooth muscle precontracted with histamine or acetylcholine was used as a tracheal smooth muscle reactivity parameter *in vitro.*
Results: Our results showed that quercetin (20 mg/kg) caused significant bronchodilation, both *in vivo* and *in vitro.*
Conclusion: Quercetin proved in laboratory conditions its ability to reduce hyperreactivity of airways as one of the main attribute of allergic asthma *(Fig. 2, Ref. 23).* Full Text in free PDF *www.bmj.sk.*
Key words: allergen, hyperreactivity of the airways, polyphenols, quercetin.

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements participate with by releasing chemical mediators. It is associated with airways hyperresponsiveness, variable airflow obstruction within the lung that is often reversible either spontaneously or with treatment (1).

The basis for acute treatment of asthma are still relievers, which act quickly to reverse bronchoconstriction and relieve asthma symptoms. Reliever medications are rapid- acting inhaled β_2-agonists and less effective anticholinergics. This therapy can relate to side effects such as tremor, tachycardia, dryness of the mouth and bitter taste (2, 3, 4).

There are some experimental studies that evaluated the bronchodilator effect of polyphenols in experimental model of asthma in association with the rising interest in these natural substances in the respirology field. Polyphenolic compounds (Provinol, Flavin7) or separate flavonoids (luteolin, apigenin and quercetin) and stilben (resveratrol) lead to the reduction of OVA-induced airway hyperresponsiveness (5–12). But only in case of Provinol study acute effect of polyphenolic compound on the hyperreactivity of asthmatic airways was analyzed during both *in vivo* and *in vitro* conditions. Due to this reason we tried to investigate acute quercetin effect on allergic asthma after single-dose administration itself.

Quercetin belongs to the subgroup of flavonols and is the most often found in onions, apples, grapes, red wine and green tea. This flavonoid confirmed several biological activities in the airways. In both human and animal studies reduced hyperreactivity of the asthmatic airways *in vivo* was observed with quercetin (9, 11, 13, 14). Quercetin's mechanism of action in bronchodilation was examined in an experimental study *in vitro* on rat trachea. The results have shown that the main sites of its action are postsynaptic (the muscarinic receptor on the smooth muscle cell) and presynaptic ones. Presynaptic mechanism involves partly NO production (15). Some experimental studies demonstrated its inhibitory effect on histamin release during all phases of allergic reaction, phosphodiesterase III, platelet activating factor, eosinophil, neutrophil, lymphocyte recruitment to the lung only within late-phase of asthmatic reaction as well as suppression of the eosinophil activation (9–11,16). Quercetin treatment (15–30 mg/kg) made lower activity of phospholipase A2 in bronchoalveolar lavage of guinea-pigs (9, 10). This natural flavonol reduced allergic airway inflammation and hyperresponsiveness due to the alternation of T_h1/T_h2 polarization

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through the suppression of transcription factor GATA-3 and increase of T-bet expression. For that reason it is able to regulate T_h1/T_h2 balance specific for asthma (11). Quercetin was engaged in the blockade of airway epithelial cell IL-8 and MCP-1 expression by modulation of PI 3-kinase/Akt/NF-κB pathway (13). It possesses anti-proliferative activity on the human airway smooth muscle (17).

Because of this profile of action we postulated that quercetin might have a therapeutic role in attenuating allergic asthma. Our study aimed at evaluation of potential acute bronchodilatory effect in experimental asthma after administration of the flavonoid quercetin during *in vivo* and *in vitro* conditions.

Materials and methods

Materials

Quercetin, ovalbumin (type III), histamine hydrochloride and acetylcholine were bought from Sigma Aldrich (Taufkirchen, Germany), aluminium hydroxide from AFT (Bratislava, Slovakia).

Experimental protocol

All procedures were carried out according to EU directives and reviewed by the Ethical Committee of the Comenius University. The guinea pigs (Trik) were acclimatized for one week in an animal house before the start of experiments.

Guinea pig pattern of allergic asthma

Male guinea pigs (200–250 g) were randomly divided into 3 experimental groups. Two groups were actively sensitized by allergen. There were 6 animals in each group. The suspension of 5 mg OVA and 100 mg aluminium hydroxide was used for active sensitization of guinea pigs by both subcutaneous and intraperitoneal injection on the 1st day. This suspension was administrated either subcutaneously or intraperitonealy again during following 15 days. Guinea pigs were nebulized by 1 % ovalbumin from the 16th day. The whole exposure by this allergen lasted 21 days. Animals were used for our experiment after the end of sensitization.

Experiments

Our study was performed during *in vivo* and *in vitro* laboratory conditions. The guinea pigs of the first group were not sensitized and served as the control group. The animals of the two remaining groups were sensitized by ovalbumin according to the pattern of allergic asthma. After the end of sensitization the experiments were actualized.

Changes in the reactivity airways were studied using whole body plethysmography, as the reaction on inhaled histamine in dose 10^{-6} mol.l^{-1} in comparison to inhaled 0.9 % NaCl. Quercetin suspension (20 mg/kg in 0.3 ml of 0.9 % NaCl) was administrated orally by pipette to guinea pigs of the third group 1 hour before examination of the specific airway conductance. This parameter was monitored both 1 hour and 5 hours after quercetin treatment in the third group and also in the first and second group.

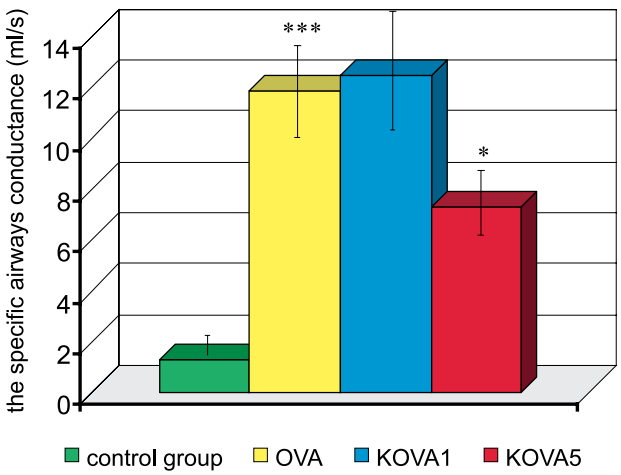

Fig. 1. The specific airways conductance of guinea pigs 21 days after sensitization by ovalbumin, 1 hour and 5 hours after oral administration of quercetin during *in vivo* conditions. Control group – nonsensitized guinea pigs by ovalbumin, without pretreatment, OVA – ovalbumin sensitized guinea pigs without pretreatment, KOVA1 – ovalbumin sensitized guinea pigs pretreated with quercetin in dose 20 mg/kg 1 hour after its oral administration, KOVA5 – ovalbumin sensitized guinea pigs pretreated with quercetin in dose 20 mg/kg 5 hours after its oral administration. Data are expressed as mean ± s.e.m., n=6, * p<0.05, *** p<0.001

Animals were killed by transversal interruption of their spinal cord 6 hours after *in vivo* study in order to acquire biological material for *in vitro* experiment. Changes in the reactivity of the tracheal smooth muscle dipped into the organ bath with Krebs-Henseleit solution were measured as the reaction on cumulative doses of bronchoconstrictor mediators histamine and acetylcholine (10^{-8}–10^{-3} mol.l^{-1}). A constant conditions were maintained (temperature, pH). Quercetin 10^{-4} mol.l^{-1} was added into organ bath with tracheal strips in the third group 30 minutes before mediators.

Statistical data analysis

Statistical significance was calculated by ANOVA. Positive differences were considered statistically significant in the cases when the p value was below 0.05. All results are expressed as mean ± s.e.m. of 6 animals.

Results

Ovalbumin caused highly significant increase of the specific airways conductance compared with control group (*** p<0.001). No differences in this parameter were measured 1 hour after oral administration of quercetin between second and third group. There was a significant bronchodilatory effect (* p<0.05) in the group treated by quercetin 5 hours after its administration compared with sensitized group without treatment *in vivo* (Fig. 1).

Allergen ovalbumin evoked a significant increase of histamine and acetylcholine induced the amplitude of tracheal smooth muscle contraction in comparison with the control. Quercetin relaxed tracheal smooth muscle precontracted with Hi (Fig. 2 A)

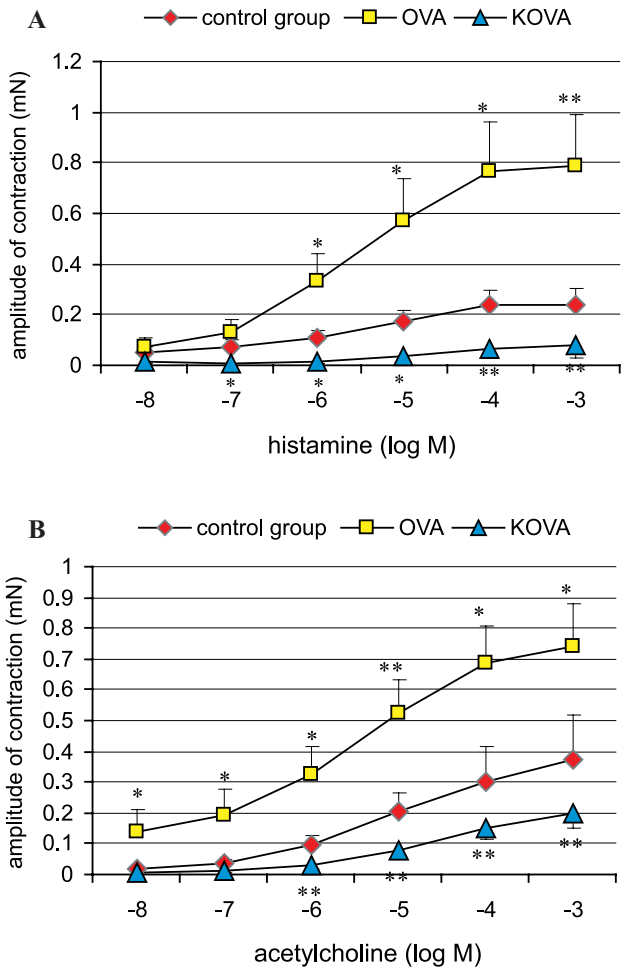

Fig. 2. The amplitude of tracheal smooth muscle contraction (mN) in ovalbumin sensitized guinea pigs as response to bronchoconstrictor mediator Hi 10^{-8}- 10^{-3} mol.l^{-1} (A) and ACh 10^{-8}–10^{-3} mol.l^{-1} (B) during *in vitro conditions.* Hi – histamine; Ach – acetylcholine; control group – nonsensitized guinea pigs by ovalbumin, without pretreatment; OVA – ovalbumin sensitized guinea pigs without pretreatment; KOVA – quercetin was added into the organ bath to ovalbumin sensitized guinea pigs 30 minutes before precontracting the tracheal smooth muscle by Hi or Ach. Data are expressed as mean ± s.e.m., n=6, * p<0.05, ** p<0.01.

and ACh (Fig. 2 B) in comparison with the control significantly (* p<0.05; ** p<0.01).

Discussion

The results of our experiment have shown a significant bronchodilatory effect of quercetin manifested 5 hours after flavonol oral administration whereas it was not able to affect the hyperreactivity of airways characteristic for allergic asthma prior to within an hour. During *in vitro* conditions this flavonol confirmed its relaxing effect on the tracheal smooth muscle.

The subsequent adaptive mechanisms modulated the definitive effect of quercetin could be integrated during *in vivo* conditions. In view of the fact that quercetin has pharmacokinetic problems such as low water solubility, fast metabolism as well as excretion, result in its low bioavailability (18, 19). We administrated quercetin orally as suspension with 0.9 % NaCl. It could partially influence its absorption in gastrointestinal tract although its positive effect was achieved after 5 hours. On the other hand some researchers found out that administration of quercetin requires formulation in dimethylsulfoxide (20). There is evidence of new quercetin prodrugs such as quercetin-amino acid conjugates and quercetin-dipeptide conjugates that showed remarkable increase in water solubility. These prodrugs can improve pharmacokinetic properties of this flavonoid and its bioavailability (19). Also quercetin-loaded microemulsion administered orally exhibited anti-inflammatory properties in a murine model of allergic inflammation (21).

Quercetin induced the decrease in the reactivity of the tracheal smooth muscle precontracted with both histamine and acetylcholine. Only in the case using low histamine concentrations there were no significant results. Quercetin was added into Krebs-Henseleit solution in the same concentration as the maximal inhibitory influence on the endothelium-intact aortic ring precontracted with noradrenaline was evoked (22). Some *in vitro* studies demonstrated inhibitory effect of quercetin or its derivatives on tracheal contractility (15, 23).

Quercetin during laboratory conditions proved its ability to reduce hyperreactivity of airways as one of the main attribute of allergic asthma. However this effect occurred 5 hours after quercetin oral administration in dose 20 mg/kg, but not earlier. Asthma inhaled relievers take effect during few minutes after their inhalation and their serum half-life is about 4–6 hours. It would be interesting to use quercetin as an adjuvant medicine together with rapid-acting β_2 agonists, but not anticholinergics due to its mechanism of action (15) in laboratory conditions or changing the way of administration to inhaled form in order to elicit immediate effect of quercetin. Some *in vivo* experimental studies showed that inhaled quercetin significantly decreased hyperreactivity of airways (10). Modification of a dissolving agent would lead to the increase of its oral bioavailability.

References

1. Bateman ED, Hurd SS, Barnes PJ et al. Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J 2008; 31: 143–178.

2. Scalabrin DMF, Sole D, Naspitz ChK. Efficacy and side effects of beta$_2$- agonists by inhaled route in acute asthma in children: comparison of salbutamol, terbutaline, and fenoterol. J Asthma 1996; 33 (6): 407–415.

3. Wellington K. Ipratropium bromide HFA. Treat Resp Med 2005; 4 (3): 215–220.

4. Rebuck AS, Chapman KR, Braude AC. Anticholinergic therapy of asthma. Chest 1982; 82: 55S–57S.

5. Joskova M, Franova S, Nosalova et al. A beneficial influence of Provinol on the reduction of allergen induced hyperreactivity in guinea pigs. Bratisl Lek Listy 2009; 110 (8): 454–458.

6. Franova S, Joskova M, Novakova E, Adamicova K, Sutovska M, Nosal S. Effect of Flavin7 on allergen induced hyperreactivity of airways. Eur J Med Res 2009; 14: 78–81.

7. Das M, Ram A, Ghosh B. Luteolin alleviates bronchoconstriction and airway hyperreactivity in ovalbumin sensitized mice. Inflammation Res 2003; 52: 101–106.

8. Choi JR, Lee ChM, Jung ID et al. Apigenin protects ovalbumin-induced asthma through the regulation of GATA-3 gene. Intern Immunopharmacol 2009; 9: 918–924.

9. Jung ChH, Lee JY, Cho ChH et al. Anti-asthmatic action of quercetin and rutin in conscious guinea-pigs challenged with aerosolized ovalbumin. Arch Pharm Res 2007; 30 (12): 1599–1607.

10. Moon H, Choi HH, Lee JY et al. Quercetin inhalation inhibits the asthmatic responses by exposure to aerosolized-ovalbumin in conscious guinea-pigs. Arch Pharm Res 2008; 31 (6): 771–778.

11. Park HJ, Lee ChM, Jung ID et al. Quercetin regulates T_h1/T_h2 balance in a murine model of asthma. Intern Immunopharmacol 2009; 9: 261–267.

12. Lee M, Kim S, Kwon OK et al. Anti-inflammatory and anti-asthmatic effects of resveratrol, a polyphenolic stilbene, in a mouse model of allergic asthma. Inter Immunopharmacol 2009; 9: 418–424.

13. Nanua S, Zick SM, Andrade JE et al. Quercetin blocks airway epithelial cell chemokine expression. Am J Resp Cell Mol Biol 2006; 35: 602–610.

14. Ko WC, Shih CH, Chen MC et al. Suppressive effects of 3-O-methylquercetin on ovalbumin-induced airway hyperresponsiveness. Planta Medica 2004; 70: 1123–1127.

15. Capasso R, Aviello G, Romano B et al. Inhibitory effect of quercetin on rat trachea contractility *in vitro*. J Pharm Pharmacol 2009; 61: 115–119.

16. Ko WC, Wang HL, Lei CB et al. Mechanisms of relaxant action of 3-O-methylquercetin in isolated guinea pig trachea. Planta Medica 2002; 68: 30–35.

17. Chaabi M, Freund-Michel V, Frossard N et al. Anti-proliferative effect of Euphorbia stenoclada in human airway smooth muscle cells in culture. J Ethnopharmacol 2007; 109: 134–139.

18. Boots AW, Haenen GRMM, Bast A. Health effects of quercetin: From antioxidant to nutraceutical. Eur J Pharmacol 2008; 585: 325–337.

19. Kim MK, Park K, Yeo W et al. In vitro solubility, stability and permeability of novel quercetin-amino acid conjugates. Bioorg Med Chem 2009; 17: 1164–1171.

20. Ferry DR, Smith A, Malkhandi J et al. Phase I Clinical trial of the flavonoid quercetin: pharmacokinetics and evidence for *in vivo* tyrosine kinase inhibition. Clin Cancer Res 1998; 2: 659–668.

21. Rogerio AP, Dora CL, Andrade EL et al. Anti-inflammatory effect of quercetin-loaded microemulsion in the airways allergic inflammatory model in mice. Pharmacol Res 2010; 61 (4): 288–297.

22. Chen CK, Pace-Asciak CR. Vasorelaxing activity of resveratrol and quercetin in isolated rat aorta. Gen Pharm 1996; 27 (2): 363–366.

23. Ko WC, Kuo SW, Sheu JR et al. Relaxant effects of quercetin methyl ether derivatives in isolated quinea pig trachea and their structure-activity relationships. Planta Med 1999; 65 (3): 273–275.

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