Cough Reflex Sensitivity and Fractional Exhaled Nitric Oxide in Children With Asthma

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Received March 30, 2020
Accepted October 6, 2020

Summary

Individual studies have suggested the utility of fractional exhaled nitric oxide (FeNO) measurement in detecting cough-variant asthma and eosinophilic bronchitis in patients with chronic cough. The aim of this study was to clarify a correlation of cough reflex sensitivity and fractional exhaled nitric oxide in asthmatic children. 25 children with asthma and 15 controls were submitted to cough reflex sensitivity measurement – capsaicin aerosol in doubling concentrations (from 0.61 to 1250 μmol/l) was inhaled by a single breath method. Concentrations of capsaicin causing two (C2) and five coughs (C5) were reported. Fractional exhaled nitric oxide (FeNO) measurement was included. Asthmatic children (11 boys and 14 girls, mean age 9±1 years) and control group (unconfirmed diagnosis of asthma) (6 boys and 9 girls, mean age 8±1 years) were included into the study. FeNO vs. C2 in asthma (Spearman´s rank correlation: -0.146, p=0.49); FENO vs. C5 in asthma (Spearman´s rank correlation: -0.777, p=0.71). We found that there is no correlation between cough reflex sensitivity and fractional exhaled nitric oxide either in children with asthma or in the control group.

Key words

Cough • Cough reflex sensitivity • FeNO • Children • Asthma

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Introduction

Cough is a vital protective reflex preventing aspiration and enhancing airway clearance. However, pathologically excessive and protracted cough is a common and disabling complaint (Morice *et al.* 2020). The commonly used definition of chronic cough in children is 4 weeks, although cough in children lasting 3-8 weeks has been termed prolonged acute cough (Chang and Glomb 2006, Shields *et al.* 2008). Irrespective of the exact duration, chronic cough in children is different from that in adults due to differences in the airway morphology, a higher degree of vulnerability to noxious insults, reduced control of the cough reflex and differences in maturation of the neurological and immunological system in the different pediatric age groups (Chang 2010). Chronic cough in children is best seen as a symptom of an underlying disease. Cough may be caused by excessive stimulation of a normal cough reflex such as occurs following inhalation of a foreign body or noxious vapors. However, most patients presenting with a chronic cough have features of cough reflex hypersensitivity, responding to exposure to low levels of thermal, chemical or mechanical stimulation (Morice *et al.* 2014). The cough hypersensitivity syndrome has been adopted as an overarching diagnosis with the different phenotypes dependent on the type and location of the inflammation seen. Both central and peripheral mechanisms have been postulated for cough reflex hypersensitivity (Mazzone *et*

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al. 2018).

Asthma is a clinical diagnosis. There is no agreed single diagnostic test to diagnose or exclude asthma, and because of its heterogeneous presentation opinions differ on how to describe the syndrome in patients with chronic cough. Eosinophilic inflammation may be a useful biomarker of asthmatic cough and may have utility in directing therapeutics. All adults and children with chronic cough may be assessed for eosinophilic inflammation. Sputum eosinophilia is perhaps the most accurate indicator, but is not routinely available, is time-consuming and requires expert interpretation. Exhaled nitric oxide can be used as a surrogate marker of eosinophilic airway inflammation and steroid responsiveness in classic asthma, but its role in asthma and chronic cough is questioned (Morice *et al.* 2020).

The first report on the presence of gaseous nitric oxide (NO) in exhaled human breath dates from 1993 (Borland *et al.* 1993). Four years later, it was found in higher than normal concentrations in children with asthma (Nelson *et al.* 1997), and higher still during asthma exacerbations, while it dropped rapidly following oral steroid therapy (Baraldi *et al*. 1997). As a result, the early 2000s saw a considerable number of publications exploring the relationship between fractional concentrations of exhaled nitric oxide (FeNO) and asthma. Nitric oxide (NO) may play an essential role in regulating airway function and in the pathophysiology of inflammatory airway diseases (Barnes 1995). NO is generated by NO synthase (NOS) from L-arginine *in vivo* (Kobzik *et al.* 1993). Nitric oxide in the respiratory system is produced mainly by two enzymes: constitutive nitric oxide synthase, which constantly generates low concentrations of NO, and inducible NOS (iNOS), the expression of which is prompted by various inflammatory cytokines (Turner 2015, Kim *et al.* 2016, Ferraro *et al.* 2018). High concentrations of NO may have not only beneficial functions (e.g. antibacterial, antiparasitic and antiviral), but also detrimental results, such as endotoxin shock (Kilbourn *et al.* 1990), apoptosis (Lipton *et al.* 1993), and pro-inflammatory effects (Barnes 1996, Hesse *et al.* 2004).

Exhaled nitric oxide (ENO) is at significantly elevated levels in bronchial asthma patients compared to healthy subjects (Kharitonov *et al.* 1994). Immunostaining of biopsied bronchial mucosa has shown that iNOS is generally present in much more amounts in the bronchial epithelium of bronchial asthma patients than normal subjects (Hamid *et al.* 1993). The augmentation of ENO results from increased iNOS expression in the airway of bronchial asthma patients. ENO measurements are recognized as a good surrogate marker for eosinophilic airway inflammation (Taylor *et al.* 2006). ENO levels in cough variant asthma patients were similar to those in bronchial asthma patients (De Diego *et al.* 2005).

Increased cough reflex sensitivity to inhaled capsaicin has been reported in chronic cough associated with eosinophilic airway inflammation, such as non-asthmatic eosinophilic bronchitis (Brightling *et al.* 2000), and atopic cough (Fujimura *et al.* 2000). It is still controversial in bronchial asthma (Fujimura *et al.* 1992, Doherty *et al.* 2000). No study has investigated the relationship between cough reflex sensitivity and FeNO in asthmatic children. NO is involved in both the normal cough reflex circuit and increased cough reflex sensitivity induced by allergic reaction (Hori *et al.* 2011). NO produced by iNOS might be a promoter in cough reflex sensitivity in asthmatic children which were of changed cough reflex sensitivity associated with allergic eosinophilic airway inflammation.

In this study, we hypothesized that NO produced by iNOS might be a promoter in cough reflex sensitivity, and therefore we performed a correlation between fractional nitric oxide (FeNO) and cough reflex sensitivity in healthy and asthmatic children.

Methods

Selection criteria and subjects

Children were referred to National Institute of Paediatric Tuberculosis and Respiratory Diseases, Dolny Smokovec, Slovak Republic by their pediatric pulmonologist. The inclusion criteria to enter the study were: 1) age from 8 to 12 years, 2) positive anamnesis of chronic cough, cough lasting longer than 4 weeks (Chang *et al.* 2001, Chang *et al.* 2017), 3) status without signs of acute airway inflammation and signs of respiratory disorders with exclusion of obstructive ventilatory defect verified by auscultation by pediatrician, spirometry examination by Geratherm Spirostik (Geratherm Respiratory GmbH, Germany) – baseline FEV1 was larger than 80 %, 4) good cooperation during the spirometry and cough reflex sensitivity (CRS) examination with relevant outcomes (hyporeactors were excluded), 5) diagnosed bronchial asthma based on current GINA guidelines (Bateman *et al.* 2018, Hogan and Bernstein 2019), 6) bronchodilatator treatment was discontinued 72 h before being examined. All children who met the given criteria

underwent CRS and FeNO measurements. Asthmatic children' (11 boys and 14 girls, mean age 9±1 years) were included into the study. The control group (6 boys and 9 girls, mean age 8±1 years) consisted of children with the presumed unconfirmed diagnosis of bronchial asthma.

The study was approved by the institutional Ethics Committee and was performed according to the Declaration of Helsinki. Each parent of the observed child was properly informed about the study, about the possibilities of cough treatment and was asked to sign an informed consent.

Spirometry, cough reflex sensitivity testing and FeNO measurement

All subjects underwent initial screening of their basic lung functions measured by spirometry before and after capsaicin challenge (Geratherm Spirostik; Geratherm Respiratory GmbH, Germany), to rule out airway obstruction.

CRS was assessed using capsaicin cough challenge, performed in agreement with the ERS guidelines (Morice *et al.* 2007) with modification for pediatric use (Varechova *et al.* 2008) (we used a compressed air-driven nebulizer (model 646; DeVilbiss Health Care, Inc., Somerset, PA, USA) controlled by a dosimeter (KoKo DigiDoser-Spirometer; nSpire health Inc., Louisville, CO, USA) with an inspiratory flow regulator valve added (RIFR; nSpire Health Inc., Louisville, CO, USA) to assign identical inspiratory flow rate during capsaicin inhalations in all subjects. Each subject inhaled saline randomly interposed among 12 inhalations of incremental capsaicin aerosol concentrations (0.61-1250 μmol/l). Each administration of saline and capsaicin aerosol was performed at 1 min intervals with the inhalation time set at 400 msec. The number of coughs within 30 s after aerosol administration was counted. The end-point of cough challenge was the inhalation of capsaicin concentration that provoked at least 5 coughs (C5) or when the maximum concentration of capsaicin (1250 μmol/l) was achieved. The con-centration of capsaicin causing at least two coughs was assigned as C2 and concentration of capsaicin causing at least 5 coughs was assigned as C5. For children that did no cough at any concentration of capsaicin, CRS value was assigned 1250 μmol/l.

The CRS measurement was realized as a single-dose capsaicin test. Gradually, with the increasing concentrations of capsaicin we determined sensitivity threshold of the airway nerve endings responsible for coughing.

FeNO measurement was realized by Niox Vero® (Aerocrine AB, Solna, Sweden). The examination was carried out on the basis of the ATS/ERS methodological recommendations (American Thoracic Society and European Respiratory Society, 2005).

Obtained parameters of CRS and FeNO were mutually statistically compared and relation between CRS and FeNO was statistically evaluated. The results were evaluated and interpreted for a group of patients and the control group.

Statistical analysis

Results with p-value below 0.05 were considered statistically significant. Software used: R Core Team (2015), R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria, URL – https://www.R-project.org/, R version 3.2.3, 2015-12-10) (Maechler *et al.* 2016).

Results

Asthmatic children (11 boys and 14 girls, mean age 9±1 years) and the control group (6 boys and 9 girls, mean age 8±1 years) were included into the study.

There was no correlation between FeNO and cough reflex sensitivity (C2) either in asthmatic children (Spearman´s rank correlation: -0.146, p=0.49) or in the control group (Spearman´s rank correlation: 0.013; p=0.96) (Fig. 1). We didn´t find the correlation between cough reflex sensitivity (C5) and FENO either in asthmatic children (Spearman´s rank correlation: -0.777, p=0.71) or in the control group (Spearman´s rank correlation: 0.358; p=0.18) (Fig. 2).

Discussion

The aim of this study was to clarify the correlation of cough reflex sensitivity and fractional exhaled nitric oxide in asthmatic children. We found that there is no correlation between cough reflex sensitivity and fractional exhaled nitric oxide either in the children with asthma or in the control group.

Chronic cough is a common entity in respiratory medicine. It is a complex disorder, the management of which has recently been defined in the guidelines of the European Respiratory Society (Morice *et al.* 2020). Several intrinsic and extrinsic factors can contribute to hypersensitivity in the cough reflex, and T_H2 cell-mediated

airway inflammation is one of the major triggers of this condition (Morice *et al*. 2014). The diagnostic approaches for cough-variant asthma (CVA) and eosinophilic bronchitis (EB), two common conditions with T_H2 inflammation, have been an integral part of the clinical guidelines for chronic cough (Morice *et al.* 2020). Since the discovery of the biological roles of endogenous nitric oxide (NO) and the standardization of simple analyzers of exhaled NO in the 1990s (Barnes and Belvisi 1993), fractional exhaled nitric oxide (FeNO) has been suggested as a potential biomarker for T_H2 inflammation (Alving and Malinovschi 2010). The great advantage of measuring FeNO values is that it only requires a simple, rapid, and noninvasive test, potentially enabling the test to be widely used in clinical practice. FeNO levels were significantly higher in patients with asthmatic cough than in those with nonasthmatic cough (Asano *et al.* 2017).

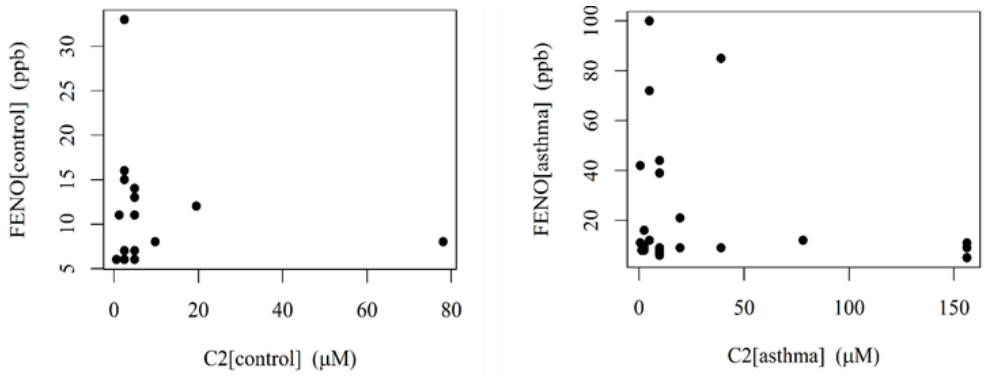

Fig. 1. Cough reflex sensitivity (C2) vs. FeNO in asthmatic children and control group. Control group – Spearman´s rank correlation: 0.013; p=0.96. Asthma group – Spearman´s rank correlation: -0.146; p=0.49.

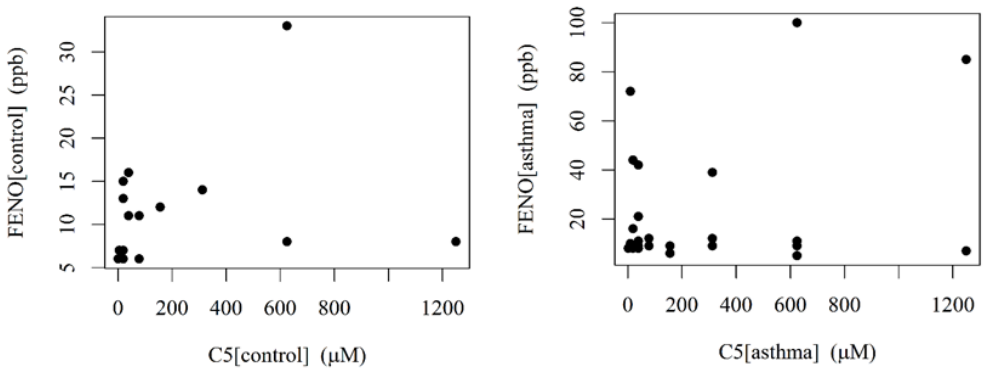

Fig. 2. Cough reflex sensitivity (C5) vs. FeNO in asthmatic children and control group. Control group – Spearman´s rank correlation: 0.358; p=0.18. Asthma group – Spearman´s rank correlation: -0.777; p=0.71.

The field of biomarker research in asthma is under continuous expansion with large-scale omics technologies facilitating the discovery of novel 'hits'. However, to date the only biomarkers in clinical use are FeNO and blood eosinophils (B-Eos) where elevated levels support ongoing type 2 inflammation and may identify candidates for novel biological treatments. FeNO can support an asthma diagnosis and has several potential roles in therapy with regard to assessing compliance and identifying candidates in need of increased inhaled corticosteroid (ICS) therapy, but also to aid down-titration of ICS, although further confirmation is required for the latter. B-Eos counts may play a role in prognosis being associated with increased exacerbations and reduced lung function. Additional clinical benefit may also be provided by the combined use of B-Eos and FeNO (Mogensen *et al.* 2020).

Non-selective NOS inhibitor L-NAME totally suppressed cough reflex sensitivity to inhaled capsaicin and reduced ENO in both non-sensitized and OVA-sensitized guinea pigs. On the other hand, although ONO1714 showed a partial suppression of cough reflex sensitivity associated with further ENO suppression in OVA-sensitized guinea pigs, it had no antitussive effect despite

ENO suppression in non-sensitized guinea pigs. ONO1714 did not influence BAL cell components 48 h after OVA challenge in sensitized animals. NO is involved in both the normal cough reflex circuit and increased cough reflex sensitivity induced by allergic reaction (Hori *et al.* 2011). However, the pathway of involvement is too complex to be explained by these results.

Limitation in this study that should be noted include the following: The unequal number of boys and girls and the sample size used in the study was rather small. Having a small sample size made the statistical results not as objective as they would be if a larger sample size had been used. In the future studies, these limitations should to be addressed.

Conclusions

We found that there is no correlation between cough reflex sensitivity and fractional exhaled nitric oxide either in children with asthma or in the control group. The precise mechanism of action need to be investigated in future studies. Possibly other biomarkers of asthma need to be added.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This work was supported by the project of Ministry of Health of the Slovak Republic – 2018/12-UKMT-8.

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