Eur Respir Rev 2006; 15: 101, 202–203
DOI: 10.1183/09059180.00010119

NONINVASIVE MARKERS OF AIRWAY INFLAMMATION AND REDOX BIOCHEMISTRY APPLIED TO ASTHMA

S. Carraro

Dept of Paediatrics, University of Padova, Padova, Italy

WINNING ABSTRACT: At the European Respiratory Society (ERS) Annual Congress 2006, I received the ERS Annual Award for Pediatric Respiratory Research in Europe for my research work on noninvasive markers of airway inflammation. Most of the studies in which I have been involved were conducted at the University of Padova, Padova, Italy. We have studied asthmatic children, and have demonstrated that some markers of inflammation (e.g cysteinyl leukotrienes) and oxidative stress (*e.g.* 8-isoprostane and malondialdehyde) are increased in their exhaled breath condensate, while antioxidant products, such as glutathione, are reduced. In addition, while I was working in the laboratory of Dr Benjamin Gaston at the University of Virginia, Charlottesville, VA, USA, I conducted some basic research studies. A first group of experiments was aimed at investigating the role of rhinovirus infection in airway acidification. A second group of experiments was conducted to investigate the mechanisms involved in the regulation of expression *S*-nitrosoglutathione reductase (GSNOR), an enzyme that has a role in asthma pathogenesis, breaking down the endogenous bronchodilator GSNO.

Silvia Carraro
Unit of Respiratory Medicine and Allergy, Dept of Paediatrics, University of Padova, Padova, Italy

MY JOB AND THE UNIT IN WHICH I WORK

I am a fifth year resident at the Dept of Paediatrics of the University of Padova, Padova, Italy. I work in the Respiratory Medicine and Allergy Unit, in the group of Prof. Eugenio Baraldi. I have also spent 1 year working in the laboratory of Prof. Benjamin Gaston, at the University of Virginia, Charlottesville, VA, USA.

MY RESEARCH AS PART OF MY WORKING GROUP/ RESEARCH TEAM

Our work in Padova (in cooperation with the National Institute of Occupational Safety and Prevention at the University of Parma, Parma, Italy) is mainly focused on the noninvasive study of airway inflammation in asthma, applying exhaled nitric oxide fraction and exhaled breath condensate (EBC) in paediatric clinical studies. EBC has been recently proposed as a noninvasive technique for the study of airway inflammation, allowing the measurement of several exhaled substances. EBC is obtained by cooling exhaled air and its composition is believed to mirror that of airway lining fluid [1]. An American Thoracic Society/European Respiratory Society Task Force has recently published a document with recommendations for the EBC technique [2]. We have conducted several studies applying the EBC method for investigating different aspects of airway inflammation in asthmatic children.

In a first group of studies, we analysed EBC for the presence of oxidative stress markers. Oxidative stress occurs when oxidant agents overcome the antioxidant defenses, resulting in tissue damage. We found that, during an acute asthma exacerbation, the lipid peroxidation marker malondialdehyde (MDA) was significantly increased, while the antioxidant glutathione was significantly reduced [3]. After a short course of oral steroids, MDA was reduced to levels similar to those detected in healthy children, while glutathione was increased but still lower than in healthy children [3]. In EBC of asthmatic children, we found increased levels of both 8-isoprostane [4], a product of the lipid peroxidation of arachidonic acid, and 3-nitrotyrosine [5], a product of the reaction between the potent oxidant peroxynitrate and a tyrosine residue. Although significantly higher in asthmatic than in healthy children, both these markers of oxidative stress were not different in children either treated or not treated with inhaled steroids [4, 5]. The observation that inhaled steroids poorly affect oxidative stress markers, suggests that antioxidants might have a role as add-on therapy in asthma management.

A second group of studies were conducted to investigate the role of cysteinyl leukotrienes (cys-LTs) as inflammatory markers in EBC of asthmatic children. We found that cys-LTs were significantly increased in asthmatic children with exercise-induced asthma [6]. Moreover, a positive correlation was demonstrated between baseline EBC cys-LT concentration and the maximum drop in forced expiratory volume in one second after the exercise test, suggesting a role for these inflammatory mediators in the pathogenesis of exercise-induced asthma [6]. In a further study, we found that in children with unstable asthma, EBC cys-LT levels were scattered over a wide range, from values similar to those detected in healthy controls to very high values, significantly higher than those detected in stable asthmatic children [7]. This observation supports the hypothesis that different

inflammatory phenotypes may underline asthmatic disease symptoms, especially in children with difficult-to-control asthma. Indeed, the measurement of cys-LT in EBC may have a role in identifying children most likely to benefit from anti-leukotriene therapy.

Finally, we have investigated EBC acidity, finding that in asthmatic subjects, EBC pH is significantly decreased [8]. In keeping with previous data, our finding supports a role for a dysregulation of pH homeostasis in asthma pathogenesis.

Besides these clinical studies, I have also conducted basic research studies while working at the University of Virginia. It is well known that acute asthma attacks are often triggered by human rhinovirus (HRV) infection, and some evidence suggests that both acute asthma exacerbation and HRV infection are associated with a significant airway acidification [9, 10]. A first group of experiments were aimed at investigating whether *in vitro* HRV infection can induce a pH fall in airway epithelial cells. The experiments demonstrated that while direct HRV infection of airway epithelial cell cultures does not decrease medium pH, a significant acidification is induced when the cells are treated with either T-helper cell (Th) type 1 cytokines or nitrosothiols, suggesting that HRV may affect pH regulatory mechanisms indirectly through the immune response [11].

A second group of experiments were aimed at investigating factors involved in regulating the expression of *S*-nitrosoglutathione reductase (GSNOR), an enzyme that seems to be important to asthma pathogenesis, breaking down the endogenous bronchodilator GSNO. A recent paper has demonstrated that mice with targeted deletion of the GSNOR gene are protected from airway bronchoconstriction [12]. Our data demonstrate that GSNOR expression is significantly increased by interleukin-13 [13]. In addition, a significant upregulation of GSNOR was described in response to treatment with steroid hormones [14].

THE IMPACT OF MY WORK ON CLINICAL OR RESEARCH PRACTICE

Taken together, both the clinical and the laboratory studies appear to be useful for the characterisation of inflammatory pathways and mechanisms involved in asthma pathophysiology. A better understanding of such processes may pave the way for more effective therapeutic strategies in the management of asthmatic patients in the future.

ACKNOWLEDGEMENTS

I would like to thank Prof. Franco Zacchello, Chief of the Dept of Paediatrics at the University of Padova, and the people that have contributed to the above-reported studies: Prof. Antonio Mutti, Dr Stefania Zanconato, Dr Massimo Corradi and Dr John Hunt.

REFERENCES

1 Hunt J. Exhaled breath condensate: an evolving tool for noninvasive evaluation of lung disease. *J Allergy Clin Immunol* 2002; 110: 28–34.
2 ATS/ERS Task Force, Horvath I, Barnes P, Hunt J. Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J* 2005; 26: 523–548.
3 Corradi M, Folesani G, Andreoli R, *et al.* Aldehydes and glutathione in exhaled breath condensate of children with asthma exacerbation. *Am J Respir Crit Care Med* 2003; 167: 395–399.
4 Baraldi E, Ghiro L, Piovan V, *et al.* Increased exhaled 8-isoprostane in childhood asthma. *Chest* 2003; 124: 25–31.
5 Baraldi E, Giordano G, Pasquale F, *et al.* 3-Nitrotyrosine, a marker of nitrosative stress, is increased in breath condensate of allergic asthmatic children. *Allergy* 2006; 61: 90–96.
6 Carraro S, Corradi M, Zanconato S, *et al.* Exhaled breath condensate cysteinyl leukotrienes are increased in children with exercise induced bronchoconstriction. *J Allergy Clin Immunol* 2005; 115: 764–770.
7 Zanconato S, Carraro S, Corradi M, *et al.* Leukotrienes and 8-isoprostane in exhaled breath condensate of children with stable and unstable asthma. *J Allergy Clin Immunol* 2004; 113: 257–263.
8 Carraro S, Folesani G, Corradi M, Zanconato S, Gaston B, Baraldi E. Acid–base equilibrium in exhaled breath condensate of allergic asthmatic children. *Allergy* 2005; 60: 206–210.
9 Hunt J, Fang K, Malik R, *et al.* Endogenous airway acidification. Implications for asthma pathophysiology. *Am J Respir Crit Care Med* 2000; 161: 694–699.
10 Ngamtrakulpanit L, Vaughan J, Nguyen A, *et al.* Exhaled breath condensate acidification during rhinovirus cold. *Am J Respir Crit Care Med* 2003; 167: 446.
11 Carraro S, Doherty J, Zaman K, *et al. S*-Nitrosothiols regulate cell-surface pH buffering by airway epithelial cells during the human immune response to rhinovirus. *Am J Physiol Lung Cell Mol Physiol* 2006; 290: L827–L832.
12 Que L, Liu L, Yan Y, *et al.* Protection from experimental asthma by an endogenous bronchodilator. *Science* 2005; 308: 1618–1621.
13 Carraro S, Palmer L, DeRonde K, *et al.* Upregulation of the asthma enzyme, *S*-nitrosoglutathione reductase, by interleukin 13. *Proc Am Thorac Soc* 2006; 3: A586.
14 Carraro S, Palmer LA, Brown-Steinke K, *et al.* Estrogen increases airway epithelial expression of the asthma enzyme, *S*-nitrosoglutathione reductase. *Proc Am Thorac Soc* 2006; 3: A424.