ELSEVIER

European Journal of Pharmacology 365 (1999) R1–R3

ejp

Rapid communication

Altered ratio of endothelin ET_A- and ET_B receptor mRNA in bronchial biopsies from patients with asthma and chronic airway obstruction

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Received 10 November 1998; accepted 20 November 1998

Abstract

Using a reverse transcription–polymerase chain reaction (RT-PCR) based assay the ratio of mRNA for the human endothelin ET_A and ET_B receptors in bronchial biopsies was assessed. In patients with diagnoses like bronchial cancer, endothelin ET_A mRNA was the dominating subtype (ratio 3.74 ± 0.99). Subjects with the diagnosis of asthma or chronic obstructive pulmonary disease showed significantly higher levels (ratio 0.81 ± 0.04) of endothelin ET_B receptor mRNA compared to endothelin ET_A receptor mRNA. Our results indicate alterations in the endothelin receptor balance in these states.

Keywords: Bronchial biopsy; Pulmonary disease; Endothelin receptor subtype

Asthma and chronic airway obstruction are important diseases of the respiratory tract in the western world. Several lines of evidence indicate an important role for endothelin in the pulmonary system and the pathogenesis of asthma (for review see Michael and Markewitz, 1996). The endothelin system consists of three isopeptides (endothelin-1, 2 and 3) and at least two subtypes of receptors (endothelin ET_A and ET_B). Endothelin-1 has been reported to be a potent constrictor in human bronchi and to act as a stimulating agent for the growth of airway smooth muscle cells in culture. In addition, increased concentrations of endothelin-1 have been found in the bronchial lavage fluid of asthmatic patients (Sofia et al., 1993) and it has been shown that the bronchial epithelial cells of patients with asthma secrete endothelin-1 (Redington et al., 1997). As little is known about the receptor balance of the endothelin-receptors in the upper part of the human bronchial tree, the aim of the present study was to investigate in bronchial biopsies whether inflammatory pathological states like asthma or chronic obstructive pulmonary disease could be correlated with alterations of the ratio of endothelin ET_A and ET_B receptor mRNA.

Bronchial biopsies were obtained from patients undergoing routine fiber-optic bronchoscopy for diagnostic reasons ($n = 13$; 6 males, 7 females; 10 smokers, 3 nonsmokers; age 61 ± 10 years). The protocol was approved by the Ethical Committee of Lund University Hospital (Lund, Sweden). The biopsies, consisting mostly of epithelial cells, were taken from the carina or first order bronchi and snap-frozen in liquid nitrogen immediately after acquisition and stored at -80°C until analysis. Total cellular RNA was extracted using TRIzol reagent (Gibco BRL) following the suppliers instructions. The amount and purity of RNA was determined by spectrophotometry considering a ratio of $O.D._{260:280} \leq 1.6$ as pure.

Ratio- reverse transcription–polymerase chain reaction (RT-PCR) for endothelin ET_A and ET_B receptors and densitometric analysis of amplification products was performed using an optimized assay under the conditions described previously (Möller et al., 1997). Briefly, amplification products (ET_A: 302 basepairs; ET_B: 428 basepairs) were separated on an agarose gel and photographed. The pictures were digitized and analyzed densitometrically before the ratio of endothelin ET_A receptor and endothelin ET_B receptor band densities was calculated. The identity of the products was verified by restriction analysis or

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0014-2999/99/$ - see front matter
PII: S0014-2999(98)00864-4

sequencing as described previously (Möller et al., 1997), a negative control (blank) containing water instead of reverse transcriptase was included in all experiments.

Densitometric analysis of the amplification products revealed in subjects with diseases like bronchial cancer ($n = 7$, 4 females, 3 males; age 60 ± 10 years) relatively low levels of endothelin ET_B mRNA compared to endothelin ET_A (ratio 3.74 ± 0.99). Patients with diagnoses of asthma or chronic obstructive pulmonary disease ($n = 6$, 3 females, 3 males; age 61 ± 11 years) showed the reversed pattern, with significantly ($P = 0.0012$, Mann–Whitney *U*-test) higher levels of endothelin ET_B mRNA compared to endothelin ET_A (ratio 0.81 ± 0.05). No signal was observed in the negative controls. No correlation with sex, age or smoking habits was found. We conclude therefore, that epithelial cells of patients with asthma and obstructive disease show a significant increased level of endothelin ET_B mRNA relative to ET_A (Fig. 1).

In previous studies of the human peripheral lung the proportion of the smooth muscle endothelin ET_A to ET_B receptors was estimated to about 32:68 with no significant alterations in samples from asthmatic and non asthmatic patients (Knott et al., 1995). However, comparative studies in pig (Goldie et al., 1996a,b) demonstrated considerable variations of smooth muscle endothelin receptor expression in different regions of the airways: while the endothelin ET_B subtype predominated in trachea (proportions 30:70) and in peripheral lung (23:65), the pattern was reversed in bronchi (70:30). Human alveolar epithelial cells were found to express endothelin receptors in a proportion about 30:70 (Abraham et al., 1997). In the present study, we found a mRNA ratio of about 3.8 in patients with diagnoses other than asthma or obstructive disease, indicating a mRNA proportion of about 78:22. Even if it is difficult to estimate receptor levels from mRNA determinations, this discrepancy suggests a similar

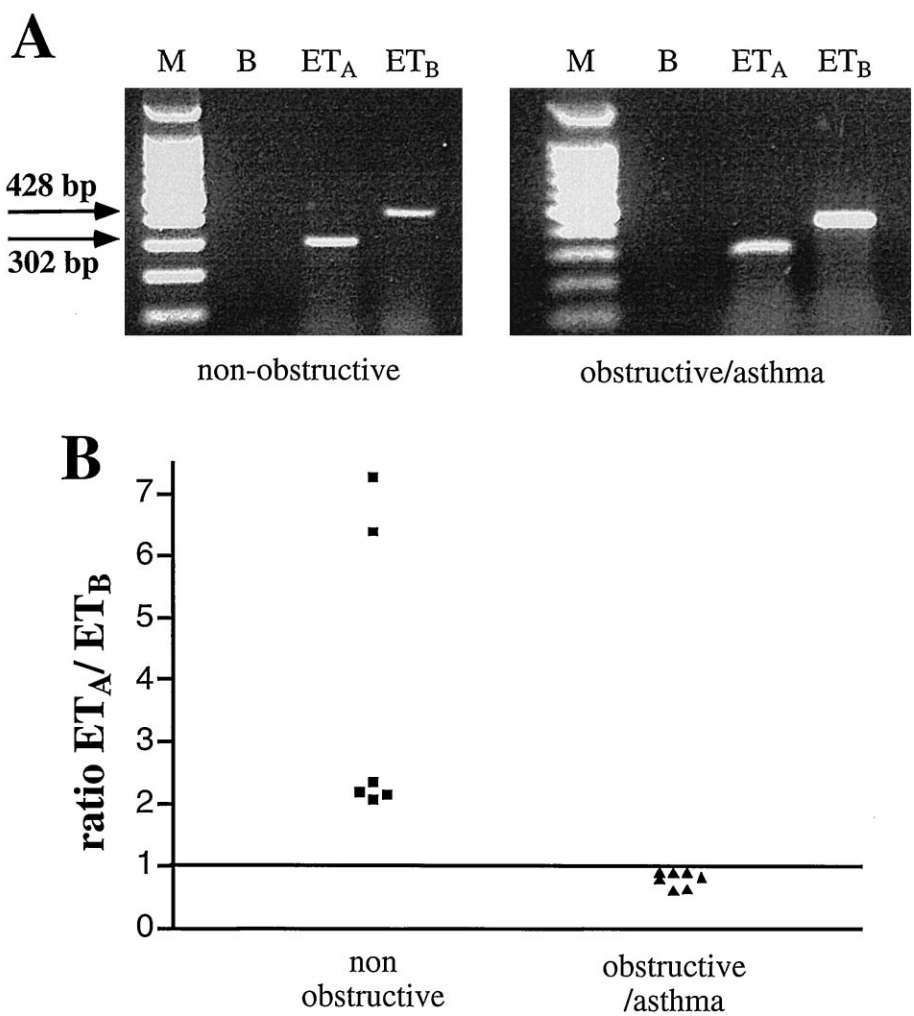

Fig. 1. (a) Representative gel electrophoresis demonstrating RT-PCR products for human endothelin ET_A (302 bp) and ET_B receptors (428 bp). The samples were obtained from subjects suffering either from bronchial cancer or chronic obstructive pulmonary disease/asthma. A 100 bp size marker (M) was used to confirm the size of the fragments. B = Blank. (b) Scatter plot of the ratio of RT-PCR products for human endothelin ET_A and ET_B receptors, sorted after diagnosis. Each point represents one patient. The horizontal line denotes ratio = 1.

regional variation of epithelial receptor expression like in smooth muscle cells. In patients with obstructive or asthmatic diseases this proportion was altered to about 45:55. In a recent study, Abraham et al. (1997) reported increased endothelin-1 expression and a comparable alteration of endothelin receptor balance in bronchial epithelial cells of patients suffering from scleroderma-associated fibrotic lung disease. This may indicate a more general role of the endothelin system in the pathophysiology of bronchial disease. To our knowledge the data presented here suggest for the first time alterations in the endothelin receptor balance in subjects with diagnosis of chronic obstructive pulmonary disease or asthma compared to other states of pulmonary disease. Further studies on the balance and regulation of the endothelin receptors in the bronchial tree will increase our understanding of their role in lung disease and hopefully provide a new rationale for optimal treatment.

Acknowledgements

The present work was supported by grants from the Swedish Medical Reasearch Council (grant no 5958), the Royal Physiografic Society, the Hulda Almroth Foundation, the Swedish Heart Lung Foundation and the Swedish Association for Allergology.

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