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Inhibitory effects of Montelukast on mediator release by nasal epithelial cells from asthmatic subjects with or without allergic rhinitis

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Summary
Aims: This study tested inhibitory effects of in vitro Montelukast treatment on nasal airway epithelial cells (AEC) cultured from asthmatic patients treated with Montelukast with and without concomitant allergic rhinitis. We further examined the effect of Montelukast withdrawal in these patients on cytokine release from cultured nasal AEC.
Methods: Nasal AEC were collected by brushings from subjects with a history of stable (no exacerbations or change in medication for $\geq$1 month) physician confirmed mild/moderate asthma whose asthma symptoms were documented to benefit from Montelukast treatment (NCT01230437). Release of the following mediators by nasal AEC were measured: IL-8, IL-6, IL-10, GM-CSF, RANTES, eotaxin and IFN-γ. Nasal AEC were cultured before and one week after withdrawal of their Montelukast treatment.
Results: Forty two asthmatics were recruited. Nasal AEC were successfully cultured in 17 at the first assessment, 14 at the second assessment and in 10 individuals at both assessments. Nasal AEC release was no different between asthmatics with or without allergic rhinitis. Montelukast significantly suppressed the release of IL-8 ($p = 0.016$), IL-6 ($p = 0.006$), RANTES ($p = 0.002$) and IFN-γ ($p = 0.046$), in a dose dependent manner in unstimulated cultures but not in those stimulated with IL-1/TNF. Withdrawal of Montelukast treatment, was associated with increased IL-8 and RANTES secretion in unstimulated nasal AEC cultured from subjects with asthma and allergic rhinitis but not with asthma alone.

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Conclusions: Montelukast treatment for asthma symptoms reversibly suppresses nasal AEC release of pro-inflammatory mediators (i.e. IL-8 and RANTES) but only in those cells cultured from subjects with concomitant allergic rhinitis.

Introduction

Asthma is a chronic inflammatory condition of the airways characterised by histopathological changes in the epithelium and submucosa. Histological alterations in the asthmatic airway include goblet cell metaplasia, squamous metaplasia, thickening of the lamina reticularis and an accumulation of both subepithelial and intraepithelial inflammatory cells [1]. Increasingly strong evidence indicates that the airway epithelium serves as a key orchestrator of the events leading to asthma [2]. Production of cytokines by the epithelium has been of particular interest with regard to allergic and asthmatic inflammation as these factors can influence infiltrating pro-inflammatory cells such as eosinophils, T-lymphocytes and mast cells [3] and also contribute to airway remodelling [4,5]. Increased susceptibility of airway epithelial cells (AEC) to injury and impaired epithelial proliferation lead to persistent mucosal injury and cause AEC to spend longer in a repair phenotype, resulting in increased production of pro-inflammatory mediators and increased secretion of pro-fibrogenic growth factors which are capable of inducing proliferation of subepithelial fibroblasts and their differentiation into activated myofibroblasts [1]. Furthermore, genetic studies have identified several asthma susceptibility genes suggesting a role for communication of epithelial damage to the adaptive immune system and activation of airway inflammation [6].

The cysteinyl leukotrienes (CysLTs) are potent lipid mediators implicated in the pathophysiology of asthma and allergic rhinitis (AR) whose effects include increased airway smooth muscle activity, microvascular permeability and airway mucus secretion [7]. CysLT(1) receptor antagonists such as Montelukast can be given orally as monotherapy in patients with mild persistent asthma [8], however for the most part these drugs are less effective than inhaled corticosteroids and are often used in an adjunct fashion [9]. Antagonism of the cysLT$_1$R with Montelukast has a number of anti-inflammatory effects some of which appear independent of cysLT$_1$R antagonism [10]. These include inhibition of plasma protein extravasation and eosinophil accumulation in small intra-parenchymal bronchi induced by ovalbumin challenge of sensitised guinea pigs [11] and inhibition of unstimulated and GM-CSF-stimulated eosinophil adhesion to VCAM-1 under shear stress conditions [12]. Montelukast also inhibited activation of NF-κB in a human monocyte/macrophage cell line [13] and suppressed IL-8 gene transcription and protein synthesis in a monocyte/macrophage cell line pre-treated with TNF [14].

We have developed a straightforward method for establishing primary cultures of nasal AEC from nasal brushings taken from adults [15] or children [16]. Our findings demonstrate that paired cultures of nasal and bronchial epithelial cells from these subjects are similar in size, shape and growth characteristics with a number of similarities in cytokine/chemokine secretion. We have not explored whether allergic rhinitis affects nasal AEC function and this is important in determining whether nasal AEC are a valid surrogate for bronchial AEC. The aim of the present study was to compare inflammatory cytokine production by nasal AEC cultured from asthmatic patients with and without concomitant AR and to examine whether in vitro treatment with Montelukast has any suppressive effects on mediator release from cultured nasal AEC. Montelukast is known to be effective in reducing symptoms of AR in asthmatic patients [17] and there is considerable interest in the hypothesis of the link between asthma and AR in the context of united airways disease [18,19]. The secondary outcome of the study therefore, was to examine pro-inflammatory cytokine and chemokine secretion by nasal AEC in a sub-group of Montelukast-responsive patients with asthma or asthma/AR after withdrawal of their Montelukast for one week.

Methods

Study design

Subjects with a history of stable (no exacerbations or change in medication for $\geq$1 month) physician confirmed mild/moderate asthma (Steps 1—4 of BTS/SIGN guidelines [20]) with $<$10 pack-year smoking histories were recruited from eight general practices in Grampian and the chest clinic at Aberdeen Royal Infirmary. To be included in the study participants had to be regularly prescribed Montelukast and to have had a documented beneficial response (symptoms, lung function, exacerbations) to the first prescription of Montelukast recorded in the medical notes. The presence or absence of AR was ascertained using the symptoms recommended by the ARIA 2008 Guidelines [21]. Of 47 asthmatic individuals who consented to take part in the study, 2 (4%) were withdrawn due to their smoking habit (smoking pack years $>$10). Of these 45 subjects all were willing to attend for a second visit that involved the same protocol after withdrawal of their Montelukast for one week, however, 3 did not attend visit 2 due to personal reasons or illness. Of the 42 subjects who came for both visits 1&2 (two weeks apart), all provided nasal samples at visit 1 and 41 subjects provided nasal samples at visit 2.

Asthma control was quantified using the Juniper-ACQ [22]. Spirometry was performed along with measurement of exhaled NO. Exclusion criteria were aspirin-sensitivity, previous nasal surgery or a history of bleeding diathesis, recent epistaxis or an upper respiratory tract infection within the past month. All participants provided written