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Urinary Glycosaminoglycan Levels Are Increased in Acute Severe Asthma – a Role for Eosinophil-Derived Gelatinase B?

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Introduction
Remodelling of the subepithelial matrix of the bronchi is a feature of mild to severe asthma [1]. Proteoglycans are important structural and functional components of the extracellular matrix, pericellular and basement membranes [2]. These macromolecules influence cell adhesion, migration and proliferation, properties which largely depend on the nature of the glycosaminoglycan (GAG) side chains. Degradation of proteoglycans is via the concerted action of heparanases and proteases [3], in particular neutrophil elastase and the matrix metalloproteases [4, 5]. The principal substrates for matrix metalloprotease-9 (92-kD gelatinase B) are collagen IV and V and the proteoglycans. Gelatinase B is activated by proteolytic degradation and inhibited by tissue inhibitor of metalloproteases-1 (TIMP-1). Although eosinophils from a patient with squamous cell carcinoma were reported to express gelatinase B [6], others reported little [7] or no [8] enzyme in normal eosinophils.

In a rat model of pulmonary emphysema [9], elastase instilled into the airways induced the release of the GAG side chains from alveolar heparan sulphate proteoglycan. A decrease in the GAG content of the lung was reflected in an increase in urinary GAG levels. In this study, we investigated the concentration of GAGs in the urine from patients with both acute and stable asthma for comparison with normal samples. We further sought evidence for the involvement of eosinophil-derived gelatinase B and TIMP-1 in tissue remodelling in asthma.

Materials and Methods
Urine samples were collected from normal individuals, patients with stable severe asthma and patients admitted to Southampton General Hospital for exacerbation of their asthma symptoms (mean peak flow < 45% predicted). Early morning mid-stream samples were collected and frozen at -20°C prior to analysis. Serum creatinine was measured colorimetrically by the
alkaline-picrate reaction (Sigma, St. Louis, Mo., USA). GAGs in urine were precipitated with 66% ethanol for 2 h at 4°C and redissolved in PBS containing 0.5 M NaCl for 0.5 h at 37°C. Total GAGs were measured by the dimethylmethylen blue dye-binding assay [10]. Specific GAGs in the extracts from patients with acute severe asthma were analysed by enzyme-specific degradation. Extracts were treated overnight with 100 mU chondroitinase ABC, heparitinase, heparinase (Sigma) or PBS as control. Immunohistochemical analysis of gelatinase B and TIMP-1 was performed on thin (2 µm) sections of resin-embedded tissue.

Results

Urine samples collected from patients the morning following admission with acute asthma contained significantly higher levels of GAGs (8.0; 1.25-17.0 µg/ml) than normal samples (2.23; 0.45-11.0 µg/ml) or samples from patients with stable severe asthma (1.93; 0.33-11.0 µg/ml). Normal kidney function in all subjects was indicated by serum creatinine levels in the normal range. The predominant urinary GAGs were chondroitin/dermatan sulphate, which might indicate the bronchi as the site of proteoglycan degradation [11]. On the fourth morning following the commencement of oral steroid therapy, urinary GAG levels were not lower, despite a significant improvement in lung function. Urinary GAG levels returned to normal over a 28-day follow-up period.

Immunohistochemical analysis of bronchial tissue from a patient with severe asthma revealed gelatinase-B-positive eosinophils, both in the tissue and circulation. In severe asthma, abundant gelatinase B was also associated with the matrix, presumably bound by its substrates. TIMP-1 in bronchial tissue was largely extracellular, notably associated with endothelial basement membranes, and could therefore not be co-localised with any specific cell type. TIMP-1 and gelatinase B activity in lysates of normal eosinophils was confirmed by Western blotting and gelatin zymography, respectively.

Conclusions

We conclude that gelatinase B released from tissue eosinophils potentially contributes to the degradation of matrix proteoglycans and that increased levels of GAGs in the urine following an episode of acute severe asthma may be a marker of matrix degradation in the airways.

References


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Eosinophils and Proteoglycan Degradation in Asthma
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