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

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Key Words

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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 collagens 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-picric acid 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 dimethylmethylene 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; 0-4.5 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,

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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

- Roche WR, Beasley R, Williams JH, Holgate ST: Subepithelial fibrosis in the bronchi of asthmatics: Characterisation, clinical and physiological correlations. *Lancet* 1989;i:520-524.
- Hardingham TE, Fosang AJ: Proteoglycans: Many forms and many functions. *FASEB J* 1992;6:861-870.
- Key NS, Platt JL, Vercellotti GM: Vascular endothelial cell proteoglycans are susceptible to cleavage by neutrophils. *Arterioscler Thromb* 1992;12:836-842.
- Nagase H, Barrett AJ, Woessner IF: Nomenclature and glossary of the matrix metalloproteinases. *Matrix* 1992;S 1:421-424.
- Murphy G, Cockcroft ML, Ward RV, Docherty AJP: Matrix metalloproteinase degradation of elastin, type IV collagen and proteoglycan. *Biochem J* 1992;277:277-279.
- Stähle-Bäckdahl M, Parks WC: 92-kD gelatinase is actively expressed by eosinophils and stored by neutrophils in squamous cell carcinoma. *Am J Pathol* 1993;142:995-1000.
- Herbert CA, Arthur MJP, Robinson

C: Augmentation by eosinophils of gelatinase activity in the airway mucosa: Comparative effects as a putative mediator of epithelial injury. *Br J Pharmacol* 1996;117:667-674. Birkland TP, Roby JD, Parks WC: 92-kD Gelatinase is selectively expressed in eosinophils from asthmatics. *Am J Respir Crit Care Med* 1996;153:A55.

9 van der Lest CHA, Versteeg EMM, Veerkamp JH, van Kuppevelt TH: Digestion of proteoglycans in porcine pancreatic elastase-induced emphysema in rats. *Eur Respir J* 1995;8:238-245.

Farndale RW, Buttle DJ, Barrett AJ: Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. *Biochem Biophys Acta* 1986;883: 173-177.

Schmid K, Grundboeck-Jusco J, Kimura A, Tschopp FA, Zollinger R, Binette JP, Lewis W, Hayashi S: The distribution of the glycosaminoglycans in the anatomic components of the lung and the changes in concentration of these macromolecules during development and aging. *Biochim Biophys Acta* 1982;716:178-187.

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