Inhibition of cathepsins B and L by kininogens: a molecular investigation

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Inflammations are characterized by the release of proteases that overwhelm their natural inhibitors. Cysteine cathepsins (CPs) that are implicated in bronchial tissue injuries emerge as key players in pulmonary inflammations, including cystic fibrosis. Among CPs, cathepsin L is an endopeptidase while cathepsin B also acts as a carboxypeptidase because of the presence of an additional occluding loop. In spite of kininogens (HMWK), that belong to the cystatin family, tightly inhibit CPs, they are degraded at inflammatory sites. Unexpectedly we found that both cathepsins B and L process HMWK under stoichiometric conditions. Therefore to understand such puzzling results, we explored molecular mechanisms of CP inhibition. While their initial inhibitory potential remained apparently unchanged, structural rearrangements of HMWK occurred following complexation with cathepsins and both CPs hydrolyzed it at cystatin-like interdomains. Conversely, cathepsin B, but not cathepsin L, cleaved inside the D3 cystatin domain in the close vicinity of the inhibitory domain, explaining its weaker inhibition by HMWK. Engineering of cathepsin B mutants demonstrated that the lack of a functional occluding loop, in addition to the impairment of the carboxypeptidase activity, restored an efficient inhibition (Ki values similar to cathepsin L inhibition), consistently with molecular modeling studies and docking calculations.

According to HMWK may associate with surfactant protein A (SP-A), we will now analyze consequences on inhibitory potential of HMWK, and on anti-microbial properties of SP-A, a major player of lung innate immunity. Supported by: C.N. and F.L.: equal contribution to the work. C.N. holds a PhD grant from VLM.

Voriconazole therapy in children with cystic fibrosis and steroid-dependent allergic bronchopulmonary aspergillosis: Efficacy and adverse effects

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Introduction: Treatment of ABPA is based on long term corticosteroid use. This often leads to inability to taper the high doses of corticosteroids (steroid-dependent ABPA). In such cases, it would be beneficial to use antifungal, such as voriconazole, to reduce the burden of A. fumigatus (AF).

Study objectives: 1) to evaluate the efficacy of voriconazole in patients with cystic fibrosis (CF) and ABPA, who are difficult to withdraw from prolonged (more than 8 months) courses of steroids (steroid-dependent ABPA) and 2) to monitor the adverse effects of voriconazole therapy.

Patients and Methods: Seven children (6 boys) aged 11–16 years (median 14 years) with CF and steroid-dependant ABPA were included. Total serum IgE and specific IgE on RAST against AF, and use of oral prednisolone, were determined prior and after a 6-month voriconazole course.

Results: After voriconazole course, total serum IgE fell from a median of 1001 IU/ml (range 3007–275) to 369 IU/ml (range 1379–83). Specific IgE also fell from a median of 13.5 IU/ml (25–1) to 6.2 (16–2). During voriconazole course, a reduction of prednisolone dosages from a median of 30mg every alternate day (range 45–20) to 12.5 mg (range 30–0) was accomplished. Adverse effects attributed to voriconazole were: a) rash in 6 children (86%), b) elevation of hepatic enzymes in 4 children (57%), c) visual problems in 2 patients (28.5%) and d) hair loss in 1 patient (14%). No child had any symptoms of adrenal insufficiency.

Conclusion: Voriconazole use in patients with CF and steroid-dependant ABPA may lead to serological improvement and allow tapering of steroid dosage by 60%. The adverse effects, although frequent, were transient and, in most cases, mild.

Increased allergic bronchopulmonary aspergillosis (ABPA) in CF – a result of global warming?

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Introduction: ABPA is an important complication of pulmonary disease in CF, occurring in 1–15% of patients. Some centres have reported a significant increase: reports of geographical clustering of cases suggest an increased exposure to Aspergillus fumigatus (AF). We speculated that this is due to an increase in AF, in turn due to altered climatic conditions. To look at this further, we compared immunological markers of AF exposure in our patients with local meteorological changes over the last 8 years.

Patients and Methods: Of 202 adult CF patients, we selected those 84 (mean age 29 years [range 20 to 52], 45 male) who had AF specific IgG (AfSI) above the normal range (40mg/ml), and compared these levels with data from the meteorological office (Station Number 7326; Latitude 53º20’N, Longitude 3º5’W; Grid Ref SJ 302755, Elevation 30m), for the years 2001–8.

Results: There was a significant increase in AfSI over time (r = 0.622, P < 0.001). Although there was no change in the average annual temperature (10.6°C), rainfall increased (mean change +3 cm/year) and there was a strong correlation between this and relative humidity and AfSI (r = 0.67 and 0.95 respectively, both P < 0.001). AfSI levels recorded in the Autumn and Winter months tended to be higher.

Conclusion: The results of global warming in the North West UK have resulted in a wetter, rather than a warmer climate. In turn, this appears to have produced conditions which are more suited to the growth of Aspergillus fumigatus, as witnessed by increased immunological makers to this organism in our CF patients. It is likely that ABPA will therefore increase in frequency as a result of this meteorological change.