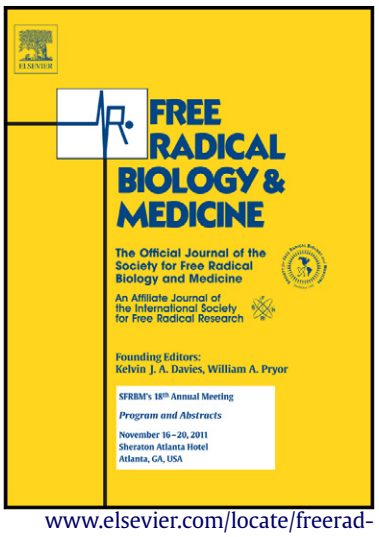

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Oxidation of Calprotectin by Hypochlorous Acid Prevents Chelation of Essential Metal Ions and Allows Bacterial Growth: Relevance to Infections in Cystic Fibrosis

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ABSTRACT

Calprotectin provides nutritional immunity by sequestering manganese and zinc ions. It is abundant in the lungs of patients with cystic fibrosis but fails to prevent their recurrent infections. Calprotectin is a major protein of neutrophils and composed of two monomers, S100A8 and S100A9. We show that the ability of calprotectin to limit growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* is exquisitely sensitive to oxidation by hypochlorous acid. The N-terminal cysteine residue on S100A9 was highly susceptible to oxidation which resulted in cross-linking of the protein monomers. The N-terminal methionine of S100A8 was also readily oxidized by hypochlorous acid, forming both methionine sulfoxide and the unique product dehydromethionine. Isolated human neutrophils formed these modifications on calprotectin when their myeloperoxidase generated hypochlorous acid. Up to 90% of the N-terminal amine on S100A8 in bronchoalveolar lavage fluid from young children with cystic fibrosis was oxidized. Oxidized calprotectin was higher in children with cystic fibrosis compared to disease controls, and further elevated in those patients with infections. Our data suggest that oxidative stress associated with inflammation in cystic fibrosis will stop metal sequestration by calprotectin. Consequently, strategies aimed at blocking extracellular myeloperoxidase activity should enable calprotectin to provide nutritional immunity within the airways.

Keywords: calprotectin, methionine, dehydromethionine, methionine sulfoxide, S100A8, S100A9, hypochlorous acid, myeloperoxidase, biomarker, inflammation, oxidative stress, nutritional immunity

Introduction

Cystic fibrosis is the most common fatal inherited disease in people of European descent [1]. It is characterized by repeated bouts of infection and inflammation in the airways [2]. The lungs of children with cystic fibrosis are initially infected with *Staphylococcus aureus* and eventually with life threatening *Pseudomonas aeruginosa* [3]. To combat infections, neutrophils swarm into the airways and attempt to phagocytose bacteria. They also release antimicrobial proteins and eject extracellular traps [4]. The most abundant protein they release is calprotectin [5]. Formally known as the cystic fibrosis antigen, calprotectin is a heterodimer of S100A8 and S100A9 [6]. Recently, calprotectin was shown to be a critical factor in innate immunity because it can prevent bacterial growth by chelating Mn and Zn ions [7]. Severe

Abbreviations: BALF, bronchoalveolar lavage fluid; MRM, multiple reaction monitoring; MS/MS, tandem mass spectrometry; ABAH, 4-aminobenzoic acid hydrazide; DPI, diphenylene iodonium