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Chapter 7

EFFECTS OF THE LACK OF THIOCYANATE IN CYSTIC FIBROSIS LUNG DISEASE

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ABSTRACT

Hypothiocyanate (OSCN-) is a major component in the lactoperoxidase system. This system in the lungs of cystic fibrosis (CF) patients is defective, which leads to bacterial colonization in the respiratory tract of these patients. Thiocyanate (SCN-) interacts with several strong oxidants, including hydrogen peroxide and hypochlorous acid, both to downregulate humoral immune response, and to create the strong antimicrobial OSCN-, in the lung. Since cystic fibrosis is characterized by recurrent respiratory infections, and these mutations of the cystic fibrosis transmembrane conductance regulator block the transport of SCN- to the respiratory tract in CF patients, it is likely that the lack of transport of SCN through the CFTR plays a central role in the pathology of this disease.

INTRODUCTION

Cystic fibrosis (CF) is an inherited disease caused by various mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) protein and characterized by chronic respiratory colonization, particularly by the bacterium Staphylococcus aureus (SA) and Pseudomonas aeruginosa (PA). Recently, it has been suggested that a lack of secretion of thiocyanate (SCN-), a component of the lactoperoxidase (LPO) system of airway defense, to the airway mucosal surfaces, is central to the colonization of these pathogens in CF airways [1]. More specifically, the lack of secretion of SCN- by mucosal cells is not only central to maintaining a sterile airway surface, but can well exacerbate the destructive effects of the various reactive oxygen species (ROS) that play a role in these defenses.

PEROXIDASES, HYDROGEN PEROXIDE AND THIOCYANATE IN HUMAN AIRWAYS

Peroxidases are an important component of airway defense. They act to catalyze the decomposition of hydrogen peroxide (H_2O_2) coupled to the oxidation of a variety of other compounds. LPO has been identified as the antibacterial agent in milk, saliva, and in tears. Myeloperoxidase (MPO) is a leukocyte derived enzyme which catalyzes the formation of a number of ROS. Both LPO and MPO are central to the production of compounds involved in the first line of bactericidal defense of human airways—LPO being secreted by submucosal cells and MPO derived from neutrophils.

The predominant product of the oxidation of SCN- by peroxide is hypothiocyanate (OSCN-). In order to complete the reaction that results in the bactericidal compound OSCN-, SCN- must be present to act as a substrate for LPO (or neutrophil myeloperoxidase) in the presence of H_2O_2 or the product of hypochlorous acid (HOCl) oxidation of proteins—chloramines.

All of these components are present in human airways, and particularly, the CFTR protein localizes in the tissues—the submucosal glands—whose function is central to the makeup of the airway lining fluid [2]. Geiszt et al used in situ hybridization to show high LPO expression within submucosal glands localized to the serous acini in human bronchium [3]. And, with the presence of H_2O_2 in the airways, LPO generates the production of HOCl, which oxidizes SCN- to produce OSCN-. As well, SCN- has also been shown to be an oxidation target of MPO, with nitric oxide acting as the driving force of the reaction, and its oxidation can also be completed by the reaction of H_2O_2 catalyzed by LPO [5].

SCN- also modulates the catalytic activity of the enzyme MPO and can serve as either its substrate or as an inhibitor [5]. This could account for the strong correlation between high MPO activity levels in circulating neutrophils and the increased severity of lung disease in CF patients [6]. Additionally, the lungs have been shown to preferentially express one of the main enzymatic complexes responsible for reactive oxygen species – the NADPH oxidase DUOX1-2 [3]. This enzyme can serve as the source for H2O2 supply for both LPO and MPO and, interestingly, in the milieu of the airways, these enzymes produce H_2O_2 with the necessity of superoxide dismutase, suggesting that this method of airway defense can be successful without the recruitment of phagocytes. However, it has also been suggested that there are higher levels of H_2O_2 in airway secretions of CF patients and this might be accounted for by the lack of LPO activity, since LPO consumes the majority of H_2O_2 in these secretions [7,8].

THIOCYANATE TRANSPORT

The strongly homologous Multidrug Resistance Associated Protein (MRP) has been shown to duplicate the function of the CFTR protein. Among these redundant functions, MRP has been shown to transport similar compounds such as the isothiocyanates (ITC) benzyl-ITC, allyl-ITC, phenethyl-ITC, as well as sulphoraphane [9,10]. In a study on SCN-

transport in human airway epithelial tissues, Pedemonte et al found that SCN- was transported from the basolateral to the apical cell surface by the CFTR protein via both the cyclic adenosine monophosphate and calcium regulated pathways [11]. And, it has been shown that SCN- anions enter the CFTR pore more easily and bind more strongly than chloride anions [12]. Conner et al demonstrated that SCN- was transported by human airway epithelia and that this transport was significantly decreased in CF airways [13,8]. Finally, SCN- levels have been shown to measure significantly lower in CF saliva, and remarkably lower concentrations in blood serum have been found in CF patients with severe pulmonary damage [14,15].

LACK OF BACTERICIDAL FUNCTION IN CF AIRWAYS

There are three ways which the host defense system can destroy bacteria once they are inhaled and deposited on airway surfaces: They can be either moved out via the mucociliary escalator or destroyed by the organic antibiotics in the airway surface fluid (for example, the beta defensins), or they can be killed by OSCN-. While the first two of these systems have been shown to be impaired in cystic fibrosis, it is the third which is responsible for the destruction of large quantities of these pathogens. In fact, it has been found that the bactericidal capacity of LPO generated OSCN- is much greater than that of the antimicrobial polypeptides [16,17]. OSCN-, in particular, is specifically active against both Pseudomonas aeruginosa and Staphylococcus aureus, two common pathogens found in CF airways.

INCREASED HUMORAL RESPONSE IN CF AIRWAYS

Cystic fibrosis is also characterized by an increased humoral immune response to invading pathogens. This is due, in part, by the inability of CF airways to remain sterile, without recruiting phagocytes, but it is also caused by the lack of SCN- in airway fluids. Once phagocytes are recruited to the airways, thiocyanate acts as a buffer there for the phagocytic oxidant product HOCl. Ashby et al showed that HOCl is capable of rapidly oxidizing SCN- to OSCN-. Indeed, these workers suggested that SCN- might act to scavenge almost all available HOCl- [18]. And, while HOCl is extremely toxic to mammalian cells, OSCN- is harmless to the airways, but very efficient at killing both PA and SA [19]. Without SCN- as a buffer, HOCl rapidly reacts with other compounds; particularly with proteins, creating chlorinated amines or chloramines. The preferred amino acid substrate for chloramines formation is taurine. Accordingly, Sarsat et al found that CF sputum contains high levels of both taurine and chloramines, and that they were negatively correlated, while secretions from normal subjects contained no taurine [20].

In addition, the product of this defense system deprived of SCN- has deleterious effects later on in the inflammatory cascade. For instance, chloramines also serve to activate neutrophil collagenase via oxidation of the enzyme. Although the role of neutrophil elastase in CF lung function has been much studied, collagenase has also been found to play a role in the destruction of these tissues in CF patients. This enzyme is implicated in tissue damage in

other lung disorders and has been measured at high levels in its active form in CF sputum. Power et al found that a negative relationship existed between collagenase activity and severity of disease in CF patients [21,22]. And, while taurine is the most abundant and preferred target for chloramine reaction, histamine, released by inflammatory mediators from mast cells and basophils, is also known to react with chloramines, and to cause host tissue damage [23]. Finally, there are a plethora of compounds known to react with chloramines and to further the procession in the cascade of inflammation. Indeed, it has been proposed that from hypochlorous acid comes the subsequent formation of chlorine, chloramines, hydroxyl radicals, and singlet oxygen [24]. Although these toxic agents are part of the cellular immune system, they also may attack normal tissue and contribute to the pathogenesis of the disease. One can easily see that SCN-, acting as a scavenger early in the process, before or shortly after these compounds are formed, could stem the destruction to tissue caused by these products of the MPO system, as well as damage caused further downstream in the inflammatory cascade.

SUMMARY

The oxidative host defense system is critical to maintaining a first line of defense in the airways. Lacking a basic component—SCN--in the LPO defense system, CF airways are missing the product of its oxidation—OSCN-. Without it, the oxidative host defense system breaks down. Since OSCN- is a major source of antibacterial defense in these tissues, this not only leads to a less than sterile airway and thus results in the recruitment of phagocytes, which serve to increase inflammation in the airways, but it also results in a lack of buffer for more cytotoxic elements present in the airway, such as the long-lived oxidants, chloramines, their byproducts and their precursor, HOCl. Certainly, the lack of SCN- in CF secretions is not the only lesion in the disease, but repairing this defect, either by inducing a functionally redundant protein such as the MRP-1 in these tissues, or reconstituting SCN- in the airways, via aerosolization, would be a major step towards ameliorating the pathology associated with the disease.

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