Antioxidant Therapy in Cystic Fibrosis

Subjects: Pathology

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Cystic fibrosis (CF) is an autosomal recessive genetic disorder characterized by major lung pathology including excess mucous build-up, chronic inflammation, persistent bacterial infections and progressive lung dysfunction. CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR is an ion channel protein responsible for transport of chloride ions (Cl-) across epithelial cell membranes and, hence, regulates fluid secretion and homeostasis. CFTR also facilitates the efflux of intracellular glutathione (GSH), one of the most predominant antioxidants in animal cells, with impaired function causing systemic deficiency in extracellular GSH. Excessive reactive oxygen species (ROS) produced by inflammatory immune cells and bacterial infection leads to a highly oxidative extracellular environment, which further contributes to lung damage.

Keywords: Cystic fibrosis, antioxidant, GGC, glutathione

1. Introduction

The thiol antioxidant glutathione is produced and maintained by all cells of the body in the millimolar range of concentrations. Extracellular glutathione concentrations can vary greatly, with plasma levels typically in the low micromolar range. In the epithelial lining fluid (ELF) of the lung, total glutathione can be 100-times higher than plasma concentrations, with increases occurring in response to stimuli such as pathogens [1][2]. High glutathione concentrations in the ELF, and the ability to raise them, is likely an adaptive response to protect lung epithelial cells against cytotoxic damage caused by bacterial and viral infections [2]. Transport of glutathione out of lung cells is predominantly conducted by the apically expressed cystic fibrosis transmembrane regulator (CFTR) protein [3]. CFTR, which is a member of the ATP-binding cassette (ABC) family of membrane transport proteins, forms a chloride and bicarbonate channel. CFTR maintains homeostasis and fluid secretion for many tissues and organs including the lung, intestine, and kidney, along with sweat and pancreatic ducts.

2. Oxidative stress in cystic fibrosis

Unsurprisingly, patients with cystic fibrosis (CF), which is an inherited disease caused by mutations in the CFTR gene, are characterised by a generalised systemic deficiency in extracellular glutathione and major lung function pathology [4]. CFTR dysfunction deregulates fluid secretion in the lungs, which leads to thick mucus formation and recurrent bacterial infections. Abnormally high levels of reactive oxygen species (ROS) with elevated pro-inflammatory markers are observed in the developing CF foetus even prior to exposure to microorganisms [5]. This innate oxidative state has been attributed to alterations in cellular proteostasis and the mitochondrial electron transport chain caused by deregulated processing and misfolded mutated CFTR. Release of toxins from bacteria that cause common and chronic respiratory infections such as *Pseudomonas aeruginosa* cell-surface lipopolysaccharide (LPS) further provokes an exaggerated neutrophilic inflammatory response that produces additional ROS [6]. The uncontrolled production of ROS can be detrimental through the promotion of aberrant cell signalling responses and the oxidative modification of biomolecules leading to lipid peroxidation, protein oxidation and dysfunction, and DNA strand-breaks, that together can promote loss of viability of lung epithelial cells [5].

3. Antioxidant defense

Under normal physiological conditions, ROS generation and consequently oxidative stress is controlled by various adaptive cellular stress responses including the transient formation of cytoplasmic stress granules (SGs), activation of autophagy and alterations to mitochondrial function. ROS generation is balanced by several antioxidant defence systems, which include enzymes such as superoxide dismutase (SOD), catalase, peroxiredoxins (Prx) and glutathione peroxidases (GPx), which employ GSH as a substrate to detoxify ROS [5]. However, due to compromised glutathione transport into the extracellular environment, excessive ROS and inflammation is persistent in the CF lung.

4. Thiol-based antioxidant therapeutics in cystic fibrosis

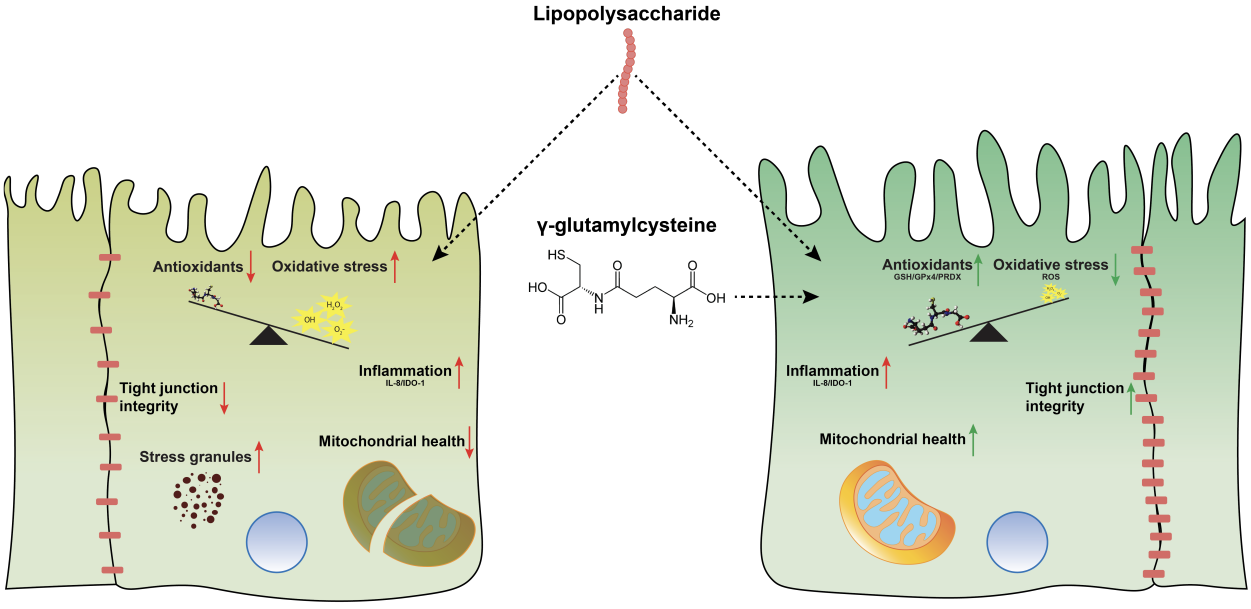
In recognition of the systemic glutathione deficiency in CF, a considerable body of research has targeted glutathione supplementation as a therapeutic strategy to restore redox homeostasis in patients with CF [4][7][8][9][10]. Glutathione is a tri-peptide composed of glutamate, cysteine and glycine that exists in a reduced (GSH) or oxidised (GSSG) state. It is synthesised in the cytosol of all cells by the activity of two enzymes: glutamate cysteine ligase (GCL) condenses glutamate and cysteine to form γ-glutamylcysteine (GGC) and glutathione synthase (GS) adds glycine to form glutathione. Homeostasis is regulated by feedback inhibition where glutathione interacts with GCL to modulate its activity.

It is thermodynamically unfeasible for exogenously administered glutathione to be passively taken up by cells due to the near 1000-fold higher total glutathione concentrations within the cell cytosol relative to that in the plasma [11]. Most cell types have a membrane bound ectoenzyme, γ-glutamyltransferase, that effectively hydrolyses glutathione to its three component amino acids during cellular uptake. This means that administered glutathione and cysteine prodrugs such as N-acetylcysteine (NAC) simply provide substrates for GCL and thereby cannot increase cellular glutathione levels above homeostasis. An inability to increase above homeostasis, may explain why studies with oral and inhaled formulations of thiols (reduced glutathione (GSH) and NAC) have repeatedly failed to demonstrate consistent improvements in CF clinical outcomes [7][8][12][13][14][15].

5. γ-glutamylcysteine as a potential therapy

Alternatively, GGC can be taken up by cells where it is converted to glutathione [16][17][18]. Furthermore, GGC itself is an antioxidant by acting as a cofactor for GPx where it is effectively comparable to GSH for hydrogen peroxide detoxification [19]. Animal safety trials have demonstrated that GGC is safe at a repeated limit dosage of 1000 mg/kg/day over a 90-day period . Exogenous supply of GGC, which is sold as a dietary supplement in the USA, has been reported to suppress oxidative injury and improve mitochondrial function both in *in vitro and in vivo* animal models of oxidative stress induced tissue damage [18][21][22][23][24]. In the context of lung disease, GGC has been tested in an LPS-induced mouse model of sepsis where it was demonstrated that GGC administration suppressed the production of LPS- induced inflammatory and oxidative mediators, which reduced lung tissue damage and reduced sepsis lethality [24]. A randomised human pilot study confirmed that single doses of orally administered GGC could significantly increase GSH levels in lymphocytes of healthy individuals within 3h, with a return to normal homeostatic levels by 5h [16]. This oral bioavailability is in part attributed to the resistance of gamma amide linkage to protease mediated hydrolysis [25].

Recently, we investigated the use of therapeutic and prophylactic GGC as a therapy for *P. aeruginosa* LPS challenge in an *in vitro* CF airway epithelium model. GGC treatment was shown to increase total intracellular glutathione levels and antioxidant protein expression and improve cell metabolic viability. In addition, epithelial barrier was improved and LPS-induced oxidative stress and stress granule formation attenuated (**Figure 1)**. While these early results are promising, defects in gut and liver function in CF patients could hamper oral GGC efficacy. Therefore, controlled clinical studies are needed to investigate the safety of GGC in CF patients and its potential as a useful preventative and therapeutic treatment for CF airway redox imbalance.



Primary Differentiated Cystic Fibrosis Human Airway Epithelial Cell

Figure 1. γ-glutamylcysteine (GGC) therapy of lipopolysaccharide (LPS) challenged cystic fibrosis human airway epithelial cells. GGC treatment increases total intracellular glutathione levels and antioxidant protein expression, improve cell metabolic viability and epithelial barriers, and attenuate LPS-induced oxidative stress and stress granule formation.

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