Clinical Trials Of Glutathione Intended To Prevent The Outcomes Of Cystic Fibrosis And The Emerging Role Of GSH Precursors In Health Care And Therapeutics

Amna Batool, Mahira Arooj

Abstract: Current treatments aimed at controlling and alleviating the fatal symptoms of cystic fibrosis (CF) have directed their attention to draw valid inferences, by employing different strategies and agents for the absolute prevention of disease. Variety of CF clinical studies exploits the master antioxidant known as glutathione (GSH) and its precursors to improve the health status of CF patients and to develop the ultimate role of therapeutics. The practical usage of GSH in various clinical trials is assessed by its ability to improve numerous clinical endpoints and surrogate markers of CF, which are yet to be determined. More recently a precursor of GSH known as Gemma-glutamylcysteine (GGC) is gaining larger acceptance, ascertained by its efficacious role in ameliorating the health status of various patients. This review has two main focuses: to identify different clinical trials elucidating the role of GSH in the etiology of CF. Secondly to unravel the beneficial effects of N-Acetylcysteine (NAC) and GGC to discern novel clinical interventions and therapies to annihilate the disastrous effects of CF successfully.

Index Terms: Cystic Fibrosis, clinical trials, Glutathione, Gemma-glutamylcysteine, N-Acetylcysteine

1 INTRODUCTION

Cystic fibrosis transmembrane conductance regulator (CFTR) gene regulates the diffusion of ions across the membrane of various epithelial tissues [1]. The dysfunction of the CFTR causes change in the electrolyte and fluid composition of different secretions thereby increasing the viscosity and ultimately fibrosis of organ [2] such as in cystic fibrosis (CF) a deadly inherited disorder. In addition to thickened mucus which is particularly present in the CF patients, a persistent chronic airway infection and inflammation in the respiratory tract due to pathogen invasion, results in excessive accumulation of active neutrophils in CF airways. The neutrophil delivers proteases such as neutrophil elastase and oxidants to kill pathogens, but it exceeds the antiprotease and antioxidant capacity of the lung resulting in oxidative stress [3], [4]. These uncontrolled proteases and reactive oxygen species (ROS) in turn start degrading immune receptors and components of extracellular matrix as well as oxidation of proteins in the airways of CF [5].

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The insufficiency of antioxidants such as glutathione (GSH) due to the mutated or dysfunctional CFTR gene also contributes towards the initiation of oxidative stress. It has been demonstrated that the excessive production of ROS is usually associated with the increased stimulation of NADPH oxidases via mediators of inflammation such as tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL1-β), xanthine oxidase and mitochondrial respiratory chain [6], [7]. Furthermore in homozygous CF individuals, the misfolded proteins accumulate in the endoplasmic reticulum (ER) and the Golgi apparatus [8] and establish interactions with the calcium dependent chaperons to cause variations in the calcium homeostasis [9]. Such anomalies give rise to ER stress and subsequently results in activation of unfolded protein response (UPR). The ER-associated degradation (ERAD) pathway in turn fails to perform its function properly and is unable to dispose of defective proteins, consequently inducing several pathways such as activation of NF-κB, ROS production and apoptosis [10], [7]. It was indicated that the proteins present in the BAL obtained from CF pediatric patients were significantly chlorinated which indicates that during inflammation the hypochlorous acid was formed considerably [11], [12].It is attributable to the fact that the neutrophils liberate hydrogen peroxide (H2O2) and superoxide and also utilizes myeloperoxidase (MPO) to convert H2O2 into wide range of ROS such as hypochlorous acid (HClO) as well as radicals including those from tyrosine and urate [13]. The HClO can also oxidize reduced form of glutathione (GSH) that neutralizes ROS, to oxidized GSH (GSSG) form [14], [15]. There are certain markers of oxidative stress that aggravate during CF conditions and are used as a diagnostic tool to assess the clinical status of CF patients such as increased oxidation of DNA and plasma proteins. Oxidative modification of proteins is an effective and reliable marker of oxidative stress for instance protein carbonyls are largely produced as a result of oxidation of several side chains of amino acids including lysine, arginine, proline and threonine [16]. Moreover nucleophilic side chains of histidine, lysine, and cysteine residues introduce carbonyl groups into proteins

by means of secondary reaction with aldehydes (Acrolein, 4-hydroxy-2-nonenal) generated during lipid peroxidation or reaction with carbonyl derivatives (deoxyosones, ketoamines, ketoaldehydes) formed by the reaction of reducing sugars or their oxidation product with the lysine residues of proteins, consequently giving rise to advanced glycation end products [17], [18]. In addition, elevated levels of carbon mono oxide (CO) and nitro tyrosine; protein nitration directly via MPO forms the basis of nitro tyrosine formation in CF patients who possess low nitric oxide concentrations, can serve as a potential marker of oxidative stress occurring in CF [19]. Moreover the oxidized form of Cysteine upregulates and induce markers associated with the chronic lung disorders such as enhanced expression of matrix glycoprotein known as fibronectin implicated in respiratory diseases [20], [21], [7]. The products derived from lipid peroxidation including isoprostanes and aldehydes, such as 4-hydroxynonenal (HNE) and malondialdehyde (MDA), are gaining special attention due to their involvement in respiratory diseases [22], [23]. It was found out that the levels of F2α-isoprostane that are prostaglandin-like compounds derived from the oxidation of essential fatty acids (arachidonic acid) and are primarily used to identify the extent of oxidative stress, were significantly higher in plasma and breath condensate of CF patients [24], [25]. Similarly MDA levels usually measured as thiobarbituric acid reactive substances (TBARS) to cholesterol ratio are also increased in CF and directly correlate with the age of patients [26], [27]. Higher quantities of proinflammatory cytokines including IL-1β, IL-6, IL-8, TNF-α and potent chemoattractants of neutrophils were indicated in bronchoalveolar lavage (BAL) of CF patients, giving rise to pro-oxidants. While the levels of immunosuppressive cytokine having anti-inflammatory properties such as IL-10 is present in lower amounts or is completely absent in CF [28]. Similarly overproduction of pro-oxidants also results from the diminished levels of GSH or the redox imbalance present in the CF patients [29], [7]. An experiment was conducted in young patients to determine if oxidants are produced during CF and results showed that the levels of MPO and 3-chlorotyrosine (biomarker of hypochlorous acid) were higher as compared to the control subjects indicating the presence of pro-oxidants in CF patients [11]. Hence the redox imbalance and impaired antioxidant systems specifically the deficiency of GSH present in CF patients are principally involved in promoting and exacerbating the conditions of oxidative stress which should be precisely identified and rectified in order to minimize the harm caused by the stress. Various sampling techniques of the respiratory tract such as bronchoscopy and bronchoalveolar lavage (BAL) involve invasive procedures [30], [31] hence repeated sampling through them is challenging. Whereas exhaled breath condensate (EBC) and sputum collection are non-invasive or semi-invasive techniques that [30], [31], [32], facilitates in discerning potential biomarkers involved in the pathology of CF [33]. The antioxidant-based defense systems are classified into three major groups. One of the groups includes enzymes that perform this task such as glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase. The second group comprises redox systems including GSH/GSSG and thioredoxin/oxidized thioredoxin (Trx/ Trx-S2), in addition to various proteins like quinone reductase, heme oxygenase, iron chelators (hemosiderin, transferrin) and copper (albumin, ceruloplasmin). The third major group consists of antioxidants such as carotenoids, uric acid, vitamin C and vitamin E [34], [35], [36]. Glutathione confers the foremost line defense against ROS [37], [38]. Its deficiency and insufficiency in the CF patients is attributed to the presence of mutated CFTR protein which is involved in GSH transport. It has been indicated that GSH performs various crucial functions of the human body including inhibition of oxidation of thiol groups [39], chelation of metals, protection of various cellular constituents from oxidative damage including DNA, proteins and lipids [40], sustain proper mucus viscosity and neutralize free radicals by donating electrons. Glutathione acts as a substrate for glutathione peroxidase (GPx) which converts GSH and H2O2 into oxidized form of GSH known as glutathione disulfide (GSSG) and water. While glutathione reductase (GR) converts GSSG back to the reduced form (GSH) [7]. GSH also regulates the normal functioning of the immune system like phagocytosis, chemotaxis, relevant apoptosis, microtubule stability, oxidant burst, antigen presentation, discharge of lysosomal enzymes, cell signaling and protection against pathogens etc. [41], [42], [43], [44]. The ratio between GSH and GSSG is closely linked with the inflammation and a disturbance in a ratio, even without infection, induces nuclear factor NF-κB pathway and results in a cascade of cytokines including TNFα, IL-6, (IL)-8, IL-1a, and others [45], [46], [47], [48]. Decreased levels of GSH also exert influence on nitric oxide system, causing depletion in the levels of free nitric oxide which performs vital functions such as ell signaling, bronchodilation and bactericidal activities [49], [50], [51], [52].The ratio of GSH to GSSG hence determines the health status of an individual, higher ratio being an indication of minimal oxidative stress [53]. GSH also inhibits the oxidation and alkylation of cysteine-rich protein known as Kelch-like ECH-associated protein 1 (Keap1). During normal conditions the transcription factor NF-E2-related factor-2 (Nrf2) is bound to Keap1 residues and is usually present in the cytosol. However during oxidative stress the cysteine residues present in the Keap1 get oxidized and results in the splitting of Nrf2 from Keap1, causing the translocation of Nrf2 from the site where it binds to genes comprising antioxidant response element (ARE) [37], [7]. Activation of Nrf2 by modifying Keap1 cysteine residues can serve as a potential therapeutic strategy which can induce and up regulate the ARE to protect against ROS and xenobiotic electrophiles [54]. The normal concentration of this antioxidant in the epithelial lining fluid (ELF) is around 400 μM that is much higher than present in plasma ~100 folds [55]. The diminished levels of GSH in the CF patients have been analyzed and determined by various trials [15]. For instance Roum et al indicated low levels of GSH in the respiratory ELF obtained from CF patients as compared to the controls. They also examined the deficiency of GSH in the plasma and revealed neutrophil domination at the site of inflammation in the ELF of CF patients. Similarly Kettle et al identified lower concentration of GSH in the CF airways owing to its oxidation by hypochlorous acid [4]. Hence it is of utter importance to improve and enhance the GSH status of CF patients that will provide a permanent solution to the complications occurring in CF and presents a promising therapeutic strategy for CF subjects. To achieve this aim

various clinical trials and methodologies have been put forward that either deliver GSH directly by various means or utilize precursors of GSH i.e. Gamma-glutamylcysteine (γ-GC) or N-acetylcysteine (NAC) to induce the levels of GSH in the body. Gamma-glutamylcysteine is not only a precursor of GSH but also an effective and potent agent used to treat the variety of disorders. For instance Pocernich and Butterfield, 2012 stated the potential implications of GGC in neurodegenerative diseases [56]. Similarly Quintana-Cabrera et al, revealed the antioxidant properties of GGC irrespective of the GSH, such as the detoxification of H2O2 and superoxide anion [57]. Such examples affirm the efficacious role of GGC that can be exploited in the pathology of CF to provide a guaranteed treatment that will open new avenues in the world of medicine and health. A successful clinical trial delivers and guides the best possible way to manage a disease. It analyzes and demonstrates the effectiveness of various treatments via different clinical outcomes for improving the human health. The CF-reported outcomes that identify and discern new therapeutic strategies are well established and have three major focuses; pulmonary function, frequency of pulmonary exacerbation and the quality of life (QoL) [58], [59]. These outcomes are grouped into primary clinical endpoints and secondary clinical end points and are regarded as the markers of disease severity. They are measured with the baseline values before and after treating a patient with specific agent such as oral or inhaled GSH. FEV1 (Forced Expiratory Volume in the first second), FVC (Forced vital capacity), FEF25–75 (forced expiratory flow between 25–75%) are usually considered as primary clinical end points that are determined through spirometry in addition to peak flow indicated by peak flow meter. The results are stated as percentage of predicted values for patients having similar height, age, ethnicity, weight and sex. For instance normal values for FVC, FEV1 and FEF25–75 are approximately 80 % or greater of the predicted and the lower values (<80%) indicate the severity of lung obstruction and abnormality such as the reduced values of FEV1 and FVC indicates increased resistance of airway in the CF. In addition FEV1 and FEV1 % predicted are considered to be the key measures of pulmonary function and it has been indicated that four out of five deaths in CF is directly or indirectly linked with the loss of pulmonary function. This data suggests that FEV1 and FEV1% are the primary clinical endpoints of CF [59]. While the secondary clinical outcomes may include; patient-reported outcomes as assessed by the CF Questionnaire for quality of life, height, weight, body mass index (BMI) (indicates nutritional status and a measure of pulmonary function), stamina, BODE index (includes BMI, airflow obstruction, dyspnea and exercise capacity) [60], frequency of pulmonary exacerbation, fecal calprotectin levels (indicate gut inflammation that can disrupt intestinal function and impedes growth), C-reactive protein (CRP) (inflammation marker), White blood cells (WBCs) count, levels of Vitamin E (as an anti-oxidant), Sweat chloride (measure of CF) and alanine transaminase (ALT) levels as a measure of GI symptom etc. [61]. The Clinical markers of oxidative stress includes free and total glutathione in serum and sputum, cell count such as in sputum, cell viability i.e. in the presence or absence of GSH, protein carbonyls (change in the concentration of proteins that were carbonylated as a measure of oxidative stress), inflammatory cell count e.g. neutrophil elastase, cytokines, 8-isoprostane (markers of oxidative stress), ascorbic acid (ROS scavenger, regulate CFTR channel), myeloperoxidase, uric acid (Hyperuricaemia in CF), Prostaglandin E2 (PGE2) (inflammatory mediator), pattern of protein oxidation, immune cells [62] changes in the levels of alveolar lipid mediators, thiols (free glutathione and GSH in blood neutrophils) [63], lipid mediators (derived from the oxidation of polyunsaturated fatty acids such as leukotrienes, prostaglandins, lipoxins, resolvins, protectins etc. having implications in the immune responses and inflammation) etc. are also analyzed and calculated [64]. In order to measure secondary clinical endpoints scientists employ different procedures to obtain practical results that can be reproduced and exerted for the benefit of CF population. For instance Hartl et al used reverse phase high performance liquid chromatography (RP-HPLC) to determine the levels of GSH in the BALF [65]. Uric acid and ascorbic acid were measured by high performance liquid chromatography (HPLC) [66]. The levels of PGE2 and 8-isoprostane were calculated in BALF using specific enzyme immunoassays while the levels of 8-isoprostane in the urine were indicated by specific 8-isoprostane affinity sorbent. To determine the level of cytokines such as TNFα, MCP-1, IL-1h, IL-2, IL-10 etc. multiplexed, particle-based flow cytometric assay was being employed [62]. In addition MPO was assayed by using the method described by Suzuki et al. The pattern of BALF proteins was demonstrated through 2-D SDS–PAGE with silver staining, before and after the GSH treatment. Moreover for the differential cell count of the BALF cells, May-Grunwald-Giemsa (MGG) stained cytospins were used, while lymphocytes were determined by the method of four-color flow cytometry [67]. Yang et al used different extraction protocols for the lipid mediators along with mass spectrometric analysis, to identify broad range of proinflammatory and anti-inflammatory lipid mediators in the sputum of CF patients. For instance they extracted oxylipins from the CF sputum by liquid-liquid extraction and solid phase extraction procedures [68]. Corti et al measured thiol concentrations in the sputum using HPLC. Neutrophils were separated by the centrifugation and cell viability and its total number was determined by the Turk's staining and Trypan blue exclusion. In addition they determined protein content by employing Bradford method using Bio-Rad protein assay reagent [69]. Girón-Moreno et al used immunoturbidimetric assay to measure the CRP and illustrated the criteria of pulmonary exacerbation [70] which occurs in CF patients based on criteria defined by Fuchs et al [71]. According to it a patient has a pulmonary exacerbation if they reveal any four of the given symptoms including; loss of appetite, purulent sputum, loss of weight, coughing up of blood, shortness of breath or difficulty in breathing, changes in lung sounds, fever, radiographic indication of new infiltrates, increase in cough and expectoration and reduction in >10% in FEV1 as compared to the reference values. Calabrese et al analyzed cough using Chronic Cough Impact Questionnaire (CCIQ) [72] and the quality of life was assayed through Cystic Fibrosis Quality of Life Questionnaire (CFQoL) [73]. While H2O2 was measured by the Fenton's reaction [74], [75] in exhaled breath condensate (EBC) as well as in the serum [76]. In order to determine the percentile values of weight, height

and BMI, Visca et al used Children Growth Chart Calculator [61]. For children age <2 they employed World Health Organization growth charts while for children age ≥2 years, growth charts of Centers for Disease Control and Prevention were utilized. An important distinction lies between the weight gain and the linear growth, used as the clinical outcomes of CF. Patients can suffer from low weight and can be treated by weight-for-age related treatments at any age whereas growth as a CF end point is limited to pediatric patients having the potential of growth [59]. In addition for the evaluation and interpretation of variations that occur between the post-trial values of the CF patients and the baseline values, various statistical methods are used such as Kruskall Wallis Tests, MannWhitney nonparametric tests, GLM procedure and linear mixed model etc.

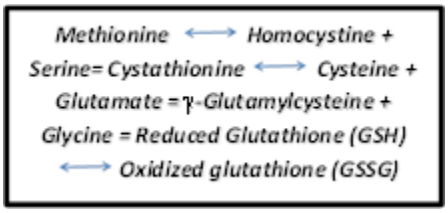

Fig. 1. *Representation of the role of Glutathione in the normal functioning of the human body.*

Selection of a suitable clinical endpoint is also a prime step and several points must be taken in to consideration before the selection process such as the timeframe of the study, intended population (age, disease severity, health), trial phase, size of the sample, availability of resources, and the aim of the study. Surrogate endpoints can be employed as a primary endpoint in phase 1 and 2 clinical trials. While the U.S. Food and Drug Administration emphasize the use of primary clinical endpoints to be used in phase 3 trials [77].

2 Clinical trials encompassing inhaled and oral administration of Glutathione (GSH) in CF patients

Many studies and trials have been conducted so far to evaluate the benefits of the inhaled and oral delivery of GSH in patients suffering from CF. These therapies have been shown to circumvent conditions and minimize damages caused as a result of oxidative stress and increase the GSH content in the human body. A conclusive role of GSH has yet to be established due to the uncertainties regarding the exact function and mechanism of GSH activity in the etiology of CF as well as the development of a suitable trial illustrating the precise administration or use of GSH for therapeutic purposes. Some of the major trials have been mentioned in this regard to explore and apprehend the ultimate goal of the glutathione in the pathophysiology of cystic fibrosis.

2.1 Oral administration of glutathione

Orally administered GSH has been utilized in few of the clinical trials to improve the GSH status and to prevent harm caused by the stresses in the CF patients.

2.1.1 Clinical Trial Kariya et al, 2007,

CFTR function assessment to boost the level of glutathione in the lung when GSH was administered orally.

Objective

The study performed by Kariya et al [78] has two main goals; to examine the glutathione levels of epithelial lining fluid of lung when GSH was given orally and secondly to determine and establish the role of CFTR in relation to it.

Study participants

This trial was conducted on mice. Wild-type male mice (C57BL/6J mice) were taken from Jackson Laboratories, another group of mice which contain S489X mutations in the murine equivalent to CFTR (C57BL/6J congenic Cftr knockout (KO) (S489X) mice) [79] and mice carrying intestinal specific expression of normal human CFTR (C57BL/6J congenic gut-corrected Cftr KO-transgenic mice) [80] were also assessed in the trial.

Procedure

Both oxidized and reduced glutathione were administered orally in wild-type mice (300 mg/kg) and were indicated in the epithelial lining fluid (ELF), lung tissues and bronchoalveolar lavage (BAL) cells after the treatment. Mice were killed after specific time intervals and plasma, BALF and lung tissues were collected after each time interval. The control mice were given PBS (125 µl) as a control vehicle. The Cftr and gut-corrected KO mice received 300 mg/kg of GSH and lung tissues as well as BALF were collected 60 min post treatment. Data was presented in the form of mean ± SE. GSH levels were determined in BALF, plasma, BAL cells and lung tissues using HPLC along with coulometric electrochemical detection.

Results

The results demonstrated that after administering the GSH orally, there was a significant increase in levels of GSH in the plasma reaching its highest level at 30 minutes post ingestion, in addition to ELF, BAL cells and lung tissues at 60 minutes. Whereas oral GSSG produced a slight increase in the level of GSH. The study thus confirmed that the oral mode of administration for GSH elevates and boosts its levels in the plasma and lung. Furthermore, to investigate the role and function of CFTR in the modulation of this process, wild-type mice were compared with Cftr knockout mice and gut-corrected Cftr knockout-transgenic mice that were given GSH orally. Same parameters were assessed including BAL cells, plasma, ELF, and lung. It was noted that the GSH levels in the wild-type mice escalated significantly up to 2-folds in plasma and lung tissues, 3-folds in BAL cells and 5-folds in the ELF at 60 minutes. However in the gut-corrected Cftr KO-Transgenic mice these levels raised up to 2-folds in the BAL cells, 40% in the plasma, 50% in the ELF and 60% in the lung. Whereas in the Cftr knockout mice, no changes in the magnitude and levels of the GSH were detected. Hence this study established a firm role of CFTR in the regulation and absorption of GSH in the body.

2.1.2 CLINICAL TRIAL, VISCA ET AL, 2015,

Oral administration of reduced L-Glutathione in a randomized clinical trial, to ameliorate growth of pediatric individuals with CF.

OBJECTIVE

The objective of the study is to analyze and determine the role of GSH in improving the growth of CF pediatric patients, when GSH was administered orally.

STUDY PARTICIPANTS

A randomized, placebo-controlled and double blind, clinical trial was performed in 44 CF pediatric patients between ages 18 months-10 years. The patients were grouped according to age and then randomly allocated to the placebo and treatment group. The criteria for CF patients to be considered in the trial include; positive for sweat chloride test (>60) or contain paired deleterious DNA CFTR mutations, age limit must be 18 months-10 years and harbor pancreatic insufficiency.

PROCEDURE

The duration of the treatment was 6 months and the blood samples were collected before and after the 6 month's trial. The placebo group received calcium citrate (65 mg/kg) and the treated group received oral GSH (65 mg/kg), thrice a day for 6 months. Primary clinical endpoints were determined including body mass index (BMI), percentile of weight and height and the gut inflammatory marker; calprotectin levels in the feces. While secondary outcome estimated inflammation markers and liver function such as levels of alanine transaminase (ALT), C-reactive protein (CRP), White blood cells (WBCs) and vitamin E.

RESULTS

During the treatment, the individuals in the GSH group gained 0.67 SD for weight-adjusted-for-age-and-sex Z score (weight percentile 19.1) as compared to the placebo 0.1 SD (weight percentile 2.1). Similarly BMI for the GSH administered group was 0.69 SD (BMI percentile 21.7) versus placebo group 0.22 SD (BMI percentile 5.2), height 0.2 SD (height percentile 7.0) while in placebo group it is −0.06 SD (height percentile −2.6), fecal calprotectin also improved in the GSH group in contrast to the placebo group (−52.0 vs 0.5, respectively). The results obtained from secondary outcomes were also encouraging. The variations occurring between the placebo and GSH group were compared with the help of 2-sample t tests. The levels of WBC and CRP were significantly reduced in the GSH administered group (WBC: −0.7, CRP: −2.6) in contrast to the placebo group (WBC: 0.6, CRP: 2.6). The mean ALT levels showed similar results with the decrease in -5.1 units in the GSH treated group and increase in 3.2 units in the placebo group. In contrast, the mean levels of vitamin E in the GSH group increased by 0.9 units vs the placebo group in which it is decreased by -0.8 units. Over all the study indicated that orally administered GSH ameliorate growth status as well as gut inflammation in CF which indicates the promise of using reduced L-GSH orally. In addition this study provided the data on the nutritional status of CF patient which was disregarded in the trials encompassing the inhalation of GSH. The GSH taken orally primarily benefit those children which are suffering from severe gut inflammation, which require the need of further study [61]. The trial however has some limitations as it included only pediatric patients (18 months-10 years) and was conducted in a single center for only six months' time period.

2.2 INHALED GSH TREATMENT

It has been noticed that the GSH in its inhaled form (nebulized or aerosolized) is more effective [81] as compared to the oral GSH in producing better results in terms of clinical endpoints as well as to increase the level of GSH in the epithelial lining fluid (ELF). Several studies have been conducted in this regard that provided an insight into the beneficial effects of inhaled GSH, forming the basis of future investigations.

2.2.1 CLINICAL TRIAL, GRIESE ET AL, 2004,

Improved and efficient administration of glutathione to boost alveolar glutathione and ameliorate lung function.

OBJECTIVE

To analyze an optimized and efficient inhalation system in order to enhance the deposition of glutathione and establish its effects on the glutathione present in the lower airways as well as on the function of lung and oxidative state.

STUDY PARTICIPANTS

Twenty one CF adolescents or adults took part in the trial out of which six CF patients were randomly assigned for the intrathoracic deposition study, with the help of a nebulizer by 99mTc-labeled Fe3O4 aerosol particles (24). Secondly seventeen subjects were selected for BAL study suffering from mild-moderate CF and having FEV1> 45% of predicted. Four patients received 300 mg doses of GSH and BAL was conducted in them after 1 hour of inhalation. While the rest of the 13 CF patients received 450 mg of GSH thrice a day, in which BAL was performed in 4 subjects after 12 hours of inhalation and in remaining 9 patients BAL assessment was made after 1 hour.

PROCEDURE

A monodisperse aerosol was produced by utilizing a Pari LC Star nebulizer that was attached to an AKITA inhalation device. This aerosol was radiolabeled (99 mTc-labeled Fe3O4 aerosol particles) [82] and is deposited and examined into the intrathoracic region of 6 CF patients. The inhalation volume was adjusted according to 75 % of inspiration capacity of the subject and the flow rate was set to 200ml per second in the course of exhalation and inhalation [83]. In addition, a BAL assessment was made in 17 other patients suffering from mild to moderate CF i.e. FEV1 > 45 % of predicted, before and after the treatment with inhaled glutathione. The treatment continued thrice a day for 2 weeks with 300-450 mg dose of GSH.

RESULTS

The levels of glutathione before and after the treatment with inhaled glutathione had no association with the lung function or the amount of leukocytes present in the BAL. It was noted that the concentration of reduced GSH in the BALF was elevated after 1 hour inhalation with either 300 or 450 mg of aerosolized GSH up to 3-4 folds respectively and the percentage of reduced GSH declined afterwards by 50-43 % respectively which implies that the administered GSH

was converted into its oxidized form. After 12 hours of inhalation the with 450 mg dose of GSH, the level of reduced GSH increased by 1.7 folds as compared to the pre-treatment level. After 2 weeks of administration with inhaled GSH, FEV1 and FVC demonstrated improvement as compared to the pre-treatment values. BAL assessment was made and it was found that total number of cells such as the number of neutrophils or proteins as well as differential cell counts did not change significantly before and after treating patients with GSH. The total amount of protein before the treatment (470 ± 175 µg/ml) and after the treatment (474 ±175 µg/ml) did not change. Similarly carbonyls (17.0 ± 1.5 pmol/µg protein) after inhalation (16.0 ± 1.3 pmol/µg protein), lipid peroxide (3.1 ± 0.4 µmol/L) after inhalation (2.8 ± 0.4 µmol/L), reduced thiols (2.0 ± 0.4 pmol -SH/µg protein) after inhalation (3.6 ± 1.1 pmol -SH/µg protein), and the concentration of neutrophils was almost similar before and after the treatment. The results indicate that by employing novel inhalation systems and approaches, the efficient deposition and the treatment of glutathione levels in the lower airways can be attained. However, the action towards oxidative stress markers present in the CF patients will require higher doses, different antioxidants and extensive treatments [84].

2.2.2 Clinical trial, Bishop et al, 2005,

A small study was conducted to evaluate the effect of buffered reduced glutathione (GSH) on the patients with CF.

Objective

To examine the role of buffer reduced inhaled glutathione on the pathophysiology of CF.

Study Participants

Trial was conducted for eight weeks on 19 individuals between age of 6-19 years and CF was diagnosed in them as a result of positive sweat chloride test. After matching age and sex, 9 patients were randomly assigned to the placebo group, while the rest 10 patients to the treatment group.

Procedure

The study was double blind, placebo and randomized controlled in which 10 patients were allocated to treatment group that receive buffered reduced GSH (66 mg/kg of body weight) and rest of the patients to the placebo group receiving NaCl with quinine (15 mg/kg; quinine: 25 to 30 g/kg). The subjects were analyzed using FEV1, forced expiratory flow at 25- 75%, FVC and peak flow. Secondary examinations include; body mass index (BMI), sputum, stamina, general health and cough frequency.

Results

The group receiving GSH fostered improved results as compared to the latter group, with no side effects. Measurement of the peak flow revealed mean change for the placebo group was 6.5 liters/minute and for the GSH group it was 33.7 liters/minute (p = 0.04). FVC, FEV1, FEF 25-75 values for the placebo group was; 87.2, 79.7 and 67.7 respectively while for GSH it was 89.3, 81.6 and 68.4. The values thus deduced from FEV1 and FVC were almost same in both groups which may be attributed to two of the given reasons; either GSH in inhaled form is not an effective way to treat CF or if GSH is potent enough than the study conducted at small scale was unable to produce detectable and desirable results. However further studies on the precise administration of the GSH will open avenues to treat pulmonary disorders effectively [52].

2.2.3 Clinical Trial, Hartl et al, 2005,

Inhalation of glutathione reduces Prostaglandin E2 and induces lymphocytes in CF patients.

Objective

To establish an association between GSH and oxidative stress, extent of lymphocytes, prostaglandin E2 and pro-inflammatory cytokines. It was hypothesized that after increasing the level of glutathione in BALF, the level of oxidative stress and inflammation decreases whereas modulation of T cell increases.

TUDY PARTICIPANTS

This trial comprises 17 CF patients with mild-moderate lung disease having median FEV1 of 67% of predicted.

Procedure

GSH was nebulized using Pari LC Star nebulizer and was administered thrice a day for 14 days with 300-450 mg dose. The lung function testing was done before administering GSH and performing BAL and after inhalation (8-12 hours) with GSH. The clinical parameters that were examined and measured included; prostaglandin E2 (PGE2), immune cells, markers of oxidative stress (myeloperoxidase, ascorbic acid, 8-isoprostane, and uric acid), pro-inflammatory cytokines, pattern of protein oxidation and total protein content as described above. BALF was assessed and data was presented in the form of mean and median by applying statistics.

Results

The inhalation of GSH caused 2-3 folds increase in GSH level of BALF and it was well tolerated by patients. Whereas the levels of PGE2 declined significantly but no significant changes were found in the levels of cytokines including IL-1h, IL-2, IL-6, IL-10, G-CSF, TNF-a, MCP-1 or MIP-1h as compared to the baseline values. It was observed that the individuals which demonstrated pronounced improvement in the lung function (FEV1) had the lowest amounts of PGE2 after the treatment. In this respect there is an inverse relation between lung function and PGE2. In contrast myeloperoxidase and 8-isoprostane showed a positive correlation with PGE2 levels in BALF. Treatment with inhaled GSH revealed no effects on the markers of oxidative stress as well as neutrophil levels present in BAL fluid. The level of 8- isoprostane also did not change when monitored in urine excretion. However the amount of lymphocytes (CD4+ and CD8+) increased remarkably, which was analyzed by flow cytometry and monoclonal antibodies. The lymphocytes showed positive correlation with the lung function. Furthermore the pattern of protein oxidation was analyzed and it was indicated that the GSH treatment did not alter the area, density and number of oxidized protein. Hence it was concluded that the fundamental role of GSH is to exert an influence on immune

responses instead of conditions of oxidative stress in the human body, requiring further examination and study [85].

2.2.4 CLINICAL TRIAL, GRIESE ET AL, 2013,

A randomized trial was held to treat patients suffering from CF with inhaled glutathione.

OBJECTIVE

To evaluate and screen glutathione through inhalation which can serve as a treatment strategy for CF.

STUDY PARTICIPANTS

The study involved 153 CF patients with FEV1 of 40–90% of predicted, and age ≥ 8 years. Patients were randomized and 73 subjects were assigned to the treated group while placebo group contained 80 patients. The mean age of the patients was 23 years and mean FEV1 % predicted was 65%. Delta-F508 mutation was present in 64% of the patients and 53% demonstrated positive culture for P. aeruginosa last year. The medications received by both the groups before and during the trial were similar, whereas GSH treated group was subjected to more anti-inflammatory treatments.

PROCEDURE

The trial was randomized, placebo-controlled and double blind to assess glutathione in the body inhaled by CF patients. The treated group received 646 mg glutathione in 4ml of water while placebo group received 0.9% NaCl in 4ml. The treatment continued for about 6 months for every 12 hours. To measure primary endpoints (FEV1), GLM method was employed for determining the covariance and for analyzing secondary clinical and laboratory endpoints (Lung function; FEV1%, FVC% and FEF25-75% of predicted, pulmonary exacerbations, weight, quality of life, changes in clinical markers; free and total GSH in sputum and serum, cell viability, total number of cells and differential cell count in sputum, inflammatory cells, sputum weight, cytokines), Mann Whitney nonparametric tests were used for the analysis of absolute changes from the basic standard. Data was presented by applying statistical analysis as mean, SD or SE.

RESULTS

The patients receiving inhaled GSH did not demonstrate a significant improvement in pulmonary symptoms, lung function or quality of life, when compared with the placebo group using CFQ-R Respiratory domain such as the occurrence of pulmonary exacerbation was similar in both groups. Moreover the GSH did not manifest any antioxidant properties against proteins or lipids and did not indicate the ability to carry out anti-proteolysis or anti-inflammation activity. The FEV1 value for the GSH group was 65.6 (14.1 SD) and for the placebo group 65.2 (14.5 SD) were almost same over six months' time period. However weight gain was observed in GSH treated group in the course of 3 months, but this effect diminished at sixth month. The pre-post differences regarding the amount of GSH in the treated group indicated an increased level of GSH as compared to the placebo group. Moreover few of the GSH metabolites like homocysteine and glutamylcysteine were lower in the treated group versus the placebo group after 1or 3 months respectively, while the levels of cysteinyl-glycine were found to be much higher. Yet cysteine was similar in both the groups in addition to the carbonylated proteins. Sputum weight, total amount of cells present in the sputum, cell count, cell differential counts neutrophil count also did not indicate any difference between both of the groups. Similarly the concentration of lipid mediators and cytokines was also same in the placebo and GSH group before and after the GSH treatment. Interestingly, cell viability in the GSH-treated group was greater, which specifies the role of GSH in the protection of cells. In addition the incidence of adverse events was alike in both of the groups. Regardless of the negative outcome obtained from the trial, it provided an in-depth study on the role of inhaled GSH in the CF individuals [63].

2.2.5 CLINICAL TRIAL, CALABRESE ET AL, 2015

Inhalation of glutathione in cystic fibrosis patients according to a randomized, single blind and placebo controlled clinical trial.

OBJECTIVE

The goal of the study is to examine and determine the effect of GSH via inhalation in CF patients. Secondly to analyze the benefits and clinical outcomes manifesting from longer treatment with GSH in adults as well as in pediatric patients.

STUDY PARTICIPANTS

The trial was single blind, randomized and placebo controlled in which both the adult patients (n=54) age ≥ 18 years and pediatric patients between age ≥ 6 to <18 years (n=51) had participated.

PROCEDURE

The inhaled GSH was given twice a day for 1 year and the assessment was made after 1,3,6,9 and 12 months of the treatment during which the patients were subjected to medical checkup and their FEV1, FVC, FEF (25-75) were also determined. Primary and secondary clinical outcomes of the subjects were measured including; body mass index (BMI), cough with the help of questionnaire (Chronic Cough Impact Questionnaire) [72], BODE index [86], stamina, quality of life monitored by questionnaire of Cystic Fibrosis Quality of Life [73], pulmonary exacerbations using Fuchs defined criteria [87], [71], C-reactive protein (CRP), hydrogen peroxide (H2O2) by utilizing Fenton's reaction [74], [75] in the serum as well as in exhaled breath condensate (EBC). In addition concomitant therapy and antibiotic therapy were being recorded.

RESULTS

No significant statistical or clinical changes were observed between the placebo group and the GSH group during or after the treatment. In pediatric patients the baseline values for the FEV1 did not change significantly in both of the groups in addition to other variables. While in adult patients the inhaled GSH for the first 9 months caused improvement in the percentage predicted FEV1, but did not persist till the end of the treatment. The inhaled GSH also did not indicate any improvement in the lung function. The inhaled GSH is

somewhat useful to the patients suffering from moderate lung disease which requires further research and study [76].

2.2.6 CLINICAL TRIAL, CORTI ET AL, 2016

The usefulness and safety of inhaled GSH therapies and examination of inflammation in vivo by setting up a murine model along with investigation of suitable alternatives.

OBJECTIVE

The aim of the study is to investigative the effectiveness of GSH inhalation. To what extent does this therapy provide any beneficial or even harmful effects. Another objective of the study is to determine the hypothesis that elevated levels of γ-glutamyltransferase (GGT) produced by inflammatory cells, are responsible for the degradation of glutathione in CF.

STUDY PARTICIPANTS

Sputum of CF patients receiving inhaled GSH was assessed for the levels of GGT as well as biological responses were observed according to the activity of GGT.

PROCEDURE

The mutated cell lines of CFTR were utilized for the in vitro experimentation. A CF mouse model was set up and transgenization of it was done by delivering human IL-8 promoter/luciferase reporter gene.

RESULTS

The results obtained by analyzing approximately 190 samples demonstrated that varied levels of GGT are present in the sputum of CF patients. The assessment of GGT activity showed that it correlates with the inflammation caused by neutrophils (neutrophil elastase) and the products obtained by the degradation of GSH via GGT. Furthermore the GSH treatment markedly decreased the levels of TNF-α, IL-1β and IL-8 in the sputum of individuals exhibiting inflammation regression. The correlation of GGT activity with the inflammatory status of the subjects advice future studies to take it into consideration while directing anti-inflammatory treatments. It was noted that during the GSH treatment, increasing the amount of GGT was linked with increased levels of oxidative stress marker i.e. protein carbonyls. In-vivo modeling enabled to examine and study the inflammation pathway including activation of NF-κB factor whereas in-vitro studies allowed scrutinizing the role of GGT in inducing prooxidant pathways. It is worthwhile to explore the exact role of GGT in the etiology of CF [88].

2.2.7 CLINICAL TRIAL, KLARE ET AL, 2016

Biofilms of Pseudomonas aeruginosa disrupted by the glutathione showed increased impact of antibiotic and a unique transcriptome.

OBJECTIVE

To introduce novel approach to eradicate resistant biofilms of Pseudomonas aeruginosa in CF. It was noted that the redox factor pyocyanin provides integrity to the biofilms of P. aeruginosa by combining with extracellular DNA. The goal of the study is to scrutinize the effect of GSH and DNase I in disrupting the biofilms of CF isolates.

STUDY PARTICIPANTS

Biofilms were obtained from isolates of CF patients and were grown in the artificial medium of CF sputum.

PROCEDURE

All Pseudomonas aeruginosa strains (Australian epidemic strain (AES) isogens AES-1R and AES-1M) were grown and maintained in Luria-Bertani broth after inoculation with CF artificial sputum medium (ASMDM+). 2 mM GSH, 40 U DNase I, 2 mM GSH + 40 U DNase I and 1×PBS for control were added in separate plates. An antibiotic known as Ciprofloxacin ((5 g/ml) was then added in these subsets. Different assays such as antibiotic susceptibility testing, RNA-sequence analysis, quantitative PCR validation as well as quantification analysis like quantitative assay of nitrate/nitrite contents, quantification of pyoverdine activity, quantification of pyocyanin and quantification of catalase production and activity were being performed.

RESULTS

The results demonstrated that the glutathione alone (2mM) or together with DNase I disrupted and impedes the immature biofilms of P. aeruginosa strains (24 hours) significantly. In addition, mature biofilms of AES-1R (72 hours) were markedly disrupted by GSH which manifested differential expression of genes (587) as evident by RNA-sequence analysis and validated by quantitative PCR assay. There was an upregulation of different systems such as the type VI secretion system, nitrate metabolism, translational machinery and cyclic diguanylate and pyoverdine biosynthesis. The GSH and DNase I mediated disruption of biofilms increased ciprofloxacin susceptibility and effectiveness which was further improved by raising the concentration of GSH, establishing the fact that GSH along with DNase I can prove to be an effective anti-biofilm treatment. The study reported a novel transcriptome for the biofilms thus disrupted. It also illustrated that the pyocyanin strengthen and improves the biofilm formation depending upon the phenotype of the P. aeruginosa adapted from the host infection, hence proposing that the biofilms represent and indicate the biofilms produced in-vivo [89]. Similarly other studies also suggest and prove the potential role of GSH as an effective antimicrobial treatment in CF patients. D'Orazio et al showed that the extracellular glutathione inhibits and prevents the invasion of Burkholderia cenocepacia in the epithelial cells of airways to cause an inflammatory response [90]. Moreover, Zhang and Duan demonstrated that the GSH in reduced as well as oxidized form prevented the growth of Pseudomonas aeruginosa and interfered with its sensitivity to different antibiotics including streptomycin, erythromycin, ciprofloxacin, chloramphenicol, kanamycin, ampicillin, cefotaxime sodium, tetracycline, and carbenicillin [91].

TABLE 1

ORAL ADMINISTRATION OF GLUTATHIONE FOR TREATING PATIENTS SUFFERING FROM CYSTIC FIBROSIS.

Year Study name xx et al	GSH dose	Duration	No. of participants	Age	Selection criteria	Primary clinical endpoints	Secondary clinical endpoints
2015, Visca et al	65mg/kg	Thrice a day for 6 months	44	18 months-10 years	Pancreatic insufficient and have CF indicated by >60 sweat chloride test result.	Weight, height, BMI, fecal calprotectin levels.	CRP, WBCs, Vitamin E, Sweat chloride, ALT, GI symptoms.

TABLE 2

TREATMENT OF THE CYSTIC FIBROSIS PATIENTS WITH INHALED GLUTATHIONE.

Year, Study name xx et al	GSH dose	Duration	No. of participants	age	Selection criteria	Primary clinical endpoints	Secondary clinical endpoints
2004, Griese et al	300 or 450 mg	Thrice a day for 14 days	21	-	Mild to moderate CF; FEV1 > 45% of predicted	FVC, FEV1	Markers of BAL fluid i.e. number of viable cells, cell count, neutrophil concentration
2005, Bishop et al	66 mg/kg	8 weeks	19	6-19 years	Positive for sweat chloride test	FVC, FEV1, FEF25–75, Peak flow	BMI, cough, sputum, stamina.
2005, Hartl et al	300 or 450 mg	Thrice a day for 14 days	17	18–39 years	median FEV1 67% of predicted	Markers of oxidative stress i.e. 8-isoprostane, ascorbic acid, myeloperoxidase and uric acid, total protein, PGE2, pro-inflammatory cytokines, pattern of protein oxidation, immune cells.	-
2013, Griese et al	646 mg	Every 12 hours for 6 months.	153	≥8 years	FEV1 40–90% of predicted	FEV1	Lung function (FEV1%, FVC% and FEF25-75% of predicted) frequency of pulmonary exacerbation, weight, quality of life, clinical markers i.e. free and total glutathione in serum and sputum, inflammatory cells, cytokines, and sputum.
2015, Calabres-e et al	10 mg/kg	Twice a day for 12 months	105	For pediatric: ≥ 6 years for adults: ≥ 18 years.	Positive for sweat chloride test	FVC, FEV1, FEF25–75	Stamina, BMI, BODE index, cough, quality of life, pulmonary exacerbations, antibiotic courses CRP, H2O2 in EBC and serum.

3 POTENTIAL ROLE OF N-ACETYLCYSTEINE (NAC) TO AMELIORATE GSH DEFICIENCY

Several studies and trials have revealed the potential of N-Acetylcysteine in enhancing the GSH content of the human body and is gaining interest in various antioxidant replenishing treatments. NAC is a produrg of cysteine residue and is a precursor of GSH which is the fundamental antioxidant [92]. As compared to cysteine NAC is less toxic, more soluble and is at low risk of being oxidized. These properties of NAC make it a suitable candidate to be used in treatment of different diseases [93]. In CF patients, the administration of NAC decreased the concentration of neutrophil in the airways of CF as well as minimized the levels of elastase and interleukin-8 [94]. Observations obtained from NAC phase II clinical trials are further supporting the oral administration of NAC; 270 mg per day for 12 weeks, which can aid in reducing the inflammation of lung and improve its function significantly [95]. Still further investigations are needed to identify the particular mechanism and utilization of NAC by which it can produce consistent results.

4 SUCCESSFUL IMPLICATIONS OF GAMMA-GLUTAMYLCYSTEINE (Γ-GC) IN VARIOUS DISEASES

Several studies have stipulated the successful and efficacious role of Gemma-glutamylcysteine (GGC) in different diseases and further research is going on to explore more and establish the propitious effects of it. GGC is a precursor to a master antioxidant known as glutathione and is involved in the biosynthesis of GSH with the help of glutathione synthase and glycine. The potential role of γ-GC has been explored in two possible ways; either γ-GC has been investigated to improve the GSH status of GSH-deficient individuals or the remarkable properties of the γ-GC have been considered to perform variety of functions such as protection against various stresses. Soon this valuable precursor will be commercialized and utilized as an active ingredient in food supplements, cosmetics including anti-aging creams and will be routinely used in different therapeutics. Ferguson and Bridge presented in detail the potential of γ-Glutamylcysteine (γ-GC) in elevating the levels of glutathione implicated in age-related disorders and avoiding the defective or impaired glutamate cysteine ligase (GCL) activity as well as rate-limiting step of this enzyme [96]. A study conducted by Pileblad and Magnijsson in the year 1992 provided an evidence that the administration of γ-glutamylcysteine in the rat brain induced glutathione levels significantly in the brain regions [97]. Hence justifying the fact that GGC has a promising role in the future of therapeutics and medicine. Similarly group of scientists proved that the GGC has the potential to eradicate the damage caused by the oxidative stress. They examined that the concentration of the GSH increases in the cultured neurons and astrocytes when they were treated with γ-glutamylcysteine. The GGC also reduced the formation of isoprostanes produced via free radicals in the neurons as well as in the astrocytes and caused the nuclear translocation of nuclear factor-erythroid 2-related factor-2 (Nrf2) in response to the oxidative stress induced by H2O2 in the astrocytes. Furthermore the study demonstrated that injecting γ-GC intravenously to the mice raised glutathione levels in various organs including heart, lungs, liver, brain, and in muscle tissues [98]. Linking an ethyl ester to γ-Glutamylcysteine known as γ-Glutamylcysteine ethyl ester (GCEE) adds additional properties to it and allows it to pass blood brain barrier and cell membrane more conveniently. GCEE have been indicated to increase the level of glutathione in the brain regions and mitochondria and protect different cells against reactive oxygen specie and nucleophilic compounds by two mechanisms; either react directly with its cysteine residue or elevate the GSH level, such as protection of neuronal cells, synaptosomes, and mitochondria in response to peroxynitrite damage [99], [100] and has been implicated as a possible therapeutic approach for neurodegenerative disease i.e. Alzheimer disease [56]. An experiment was done by using γ-Glutamylcysteine alone (100 µmol/L) or the combination of GGC with linoleic acid (10, 50, 100 µmol/L) to modulate the oxidative stress generated in human umbilical vein endothelial cells. The duration of the trial was 24 hours and the clinical endpoints that were determined in the endothelial cells include; thiobarbituric acid reactive substances (TBARS), 8-epi-PGF2a, transcription factor, GSH, GSH synthetase expression and total antioxidants. Significant reduction in the levels of 8-epi-PGF2a and TBARS were found in the cells treated with GSH alone as compared to control cells. No changes were seen in the total antioxidant level in GSH-treated cells. Whereas the combination of GGC with 10 µmol/L of linoleic acid (CLA) resulted in higher levels of GSH, GSS protein, TBARS, and 8-epi-PGF2a in contrast to cells treated with GGC alone indicating the prooxidant effects of CLA at lower doses. However it was noted that GGC+ 50 µmol/L CLA caused reduction in the levels of TBARS similar to the GSH-treated cells [101]. A similar study was conducted by the same group of scientists in 2011 which signified the role of γ-glutamylcysteine in preventing oxidative stress and damage caused by it, occurring in human endothelial cells. Another study demonstrated that the fibroblast cultures obtained from the glutathione synthetase deficient patients have insufficient levels of GSH but exhibit accumulation of γ-GC. It was proposed that γ-GC has the potential to compensate for the GSH deficiency in conditions of oxidative stress, as it contains both the reactive groups of GSH including; gamma-glutamyl group and sulphydryl group which can partly minimize the implications caused due to low levels of GSH in the affected individuals [102]. This efficacious role of γ-GC was examined and confirmed by Quintana-Cabrera et al [57], who demonstrated that by directing γ-GC production in the mitochondria, the γ-GC can perform antioxidant functions such as detoxification of H2O2 and superoxide anion, irrespective of the GSH content present in an individual. The synthesis of γ-GC was governed in the mitochondria by knocking out glutathione peroxidase-1 that resulted in increased concentration of superoxide anions. The neuro-protective role of the γ-GC has also been explored. It was shown that γ-GC prevented neuronal loss and motor deterioration in neurodegenerative mouse models and also inhibited the apoptotic death in various neurotoxic paradigms. These potential roles of γ-GC have been suggested to occur by acting as a cofactor to a peroxidase enzyme knows as glutathione peroxidase-1. More recently scientist determined if oral administration of GGC is effective in elevating the GSH levels in cells above homeostasis, which would serve towards serious health implications caused by the diminished levels or activity of

GSH. Lymphocytes were examined and assessed for GSH levels before and after treating them with oral γ-GC (2 and 4 grams). In all of the clinical trial held in this study, there was a notable increase in GSH levels. In comparison to base line levels, 2g γ-GC was able to increase the levels of GSH in lymphocytes within 90 minutes. Pharmacokinetics parameters exhibited that both the γ-GC dosage i.e. 2 and 4 grams resulted in increased concentration of GSH; 2-folds by 2g and 3-folds by 4g of γ-GC, over 3 hours (t max), after which these levels deteriorated to baseline values by 5 hours. Hence this study proposed that directing γ-Glutamylcysteine in cells that lack sufficient GSH is a safe therapeutic strategy that can readily pass through the cell membranes and converted into the glutathione [103]. These studies highlighted the positive outcomes received as a result of GGC administration in variety of diseases. Based on this data a successful implication of this precursor is speculated for the treatment of cystic fibrosis that will transcend nearly of the CF treatments in providing the therapeutics against this deadly disorder.

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