

Expert Opinion

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Carbocysteine: clinical experience and new perspectives in the treatment of chronic inflammatory diseases

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Background: Carbocysteine is a muco-active drug with free radical scavenging and anti-inflammatory properties. It is actually approved for clinical use as adjunctive therapy of respiratory tract disorders characterized by excessive, viscous mucus, including chronic obstructive airways disease (COPD). **Objective:** The intriguing antioxidant and anti-inflammatory properties of carbocysteine, beyond its known mucolytic activity, are described to explain its therapeutic efficacy and suggest new clinical uses. **Methods:** After reviewing physiology and preclinical studies, human studies on the use of carbocysteine in chronic inflammatory diseases, i.e., COPD and cancer cachexia, are reviewed. **Results/conclusions:** Carbocysteine has been recently recognized as an effective and safe treatment for the long-term management of COPD, able to reduce the incidence of exacerbations and improve patient quality of life. Moreover, carbocysteine was effective in counteracting some symptoms associated with cancer cachexia. Preclinical and clinical studies have demonstrated that the antioxidant and anti-inflammatory properties of carbocysteine are more important than mucolysis itself for its therapeutic efficacy. Therefore, carbocysteine may be able to reverse the oxidative stress associated with several chronic inflammatory diseases, such as cardiovascular diseases and neurodegenerative disorders. Controlled, randomized studies in humans are warranted.

Keywords: cancer cachexia, carbocysteine, chronic inflammation, COPD, glutathione, oxidative stress

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1. Introduction

Carbocysteine (or S-Carboxymethylcysteine; SCMC) is a muco-active drug with free radical scavenging and anti-inflammatory properties. It is available as an oral preparation both as SCMC and its lysine salt (SCMC-lys) actually approved for clinical use as adjunctive therapy of respiratory tract disorders characterized by excessive, viscous mucus, including chronic obstructive airways disease (COPD) [1].

2. Overview

Chronic obstructive pulmonary disease (COPD) remains a major public health problem. It is the fourth leading cause of chronic morbidity and mortality in the USA, and is projected to rank fifth in 2020 in burden of disease caused worldwide, according to a study published by the World Bank/World Health Organization [2]. COPD is a preventable and treatable disease with some significant extrapulmonary effects that may contribute to its severity. Its pulmonary component

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is characterized by airflow limitation that is progressive and not fully reversible. Pathological changes characteristic of COPD are found in proximal airways, peripheral airways, lung parenchyma and pulmonary vasculature. These changes include chronic inflammation and structural changes resulting from repeated injury and repair. There is a characteristic pattern of inflammation in the lungs of COPD patients, with increased number of neutrophils, macrophages and CD8⁺ lymphocytes. Lung inflammation is further amplified by oxidative stress. Physiological changes characteristic of the disease include mucus hypersecretion, airflow limitation, air trapping and gas exchange abnormalities. It is increasingly recognized that COPD involves several systemic features, particularly in patients with severe disease, and that these have a major impact on survival and comorbid diseases. Cachexia is commonly seen in patients with severe COPD. There may be a loss of skeletal muscle mass and weakness. Patients with COPD also have increased likelihood of having osteoporosis, depression and chronic anemia. Increased concentrations of inflammatory mediators including TNF- α , IL-6 and reactive oxygen species (ROS), may mediate these systemic effects. There is, moreover, an increased risk of cardiovascular disease, which is correlated with an increase in C-reactive protein (CRP) [3].

3. Oxidative stress and inflammation: the glutathione as the main cell antioxidant

3.1 Oxidative stress

The presence of oxygen is a fundamental component of cellular metabolism. Aerobic energy metabolism, or oxidative phosphorylation, is a critical metabolic pathway within cells to provide energy. Inside the mitochondria, the electron transport is responsible for a series of redox reactions that result in the synthesis of ATP. As the demand and subsequent flux for this process increases so does the chance that redox uncoupling will occur and increase the accumulation of free radicals throughout the cell [4]. The complete reduction of oxygen can be seen from the steps outlined in Figure 1 [5].

Any situation that results in an acute or chronic overconsumption of oxygen can lead to the production of the free radicals, which are more appropriately termed ROS. A free radical is a molecule that contains at least one unpaired electron in its outer spin orbits. Superoxide radicals, hydroxyl radicals, hydrogen peroxide, nitric oxide, lipid alkoxyl and peroxy radicals are the most common ROS in living, aerobic systems. Normal human cells produce small amounts of ROS, which are reduced by antioxidant enzymes and low molecular weight radical scavenger. It is widely accepted that ROS play both positive and negative roles *in vivo*. Positive are those related to ROS involvement in energy production, phagocytosis, regulation of cell growth and intercellular signaling and synthesis of biologically active compounds [6].

To protect against the deleterious effect of ROS, our body is provided with a complex system of endogenous antioxidant

protection in the form of enzymes such as superoxide dismutase, catalase and glutathione peroxidase, as well as non-enzymatic defenses, such as GSH, the iron-binding protein transferrin, dihydrolipoic acid and reduced CoQ10 [7]. *In situations* in which the production of prooxidant molecules increases to a point where the antioxidant system cannot effectively remove them, oxidative stress is known to occur. Indeed, oxidative stress is defined as the imbalance between oxidant and antioxidants in favor of the oxidants.

ROS, if not detoxified by the antioxidant system, exert a toxic action on circulating proteins, proteins of cell surface, enzymes and nucleic acid (DNA). One of the most frequent targets is the polyunsaturated fatty acids that largely comprise the cell membranes. The systematic oxidation of these polyunsaturated fatty acids, called lipid peroxidation, is one of the primary means by which oxidative stress leads to an overall decrease of cellular functions. Thus, an adequate presence and functioning of antioxidant systems is paramount for cell activity. Vice versa, oxidative stress is implicated in a number of diseases including atherosclerosis, pulmonary fibrosis, cancer, Parkinson's disease and multiple sclerosis, and ageing [8].

It has been suggested that increasing the circulating levels of certain antioxidants (i.e., glutathione, cysteine, α -lipoic acid and Vitamins A, C and E) would help to prevent the oxidative stress. Therefore, supplementation with antioxidants, either through an increased consumption in the diet or from supplementation, is able to improve one's health [4].

3.2 Inflammation-driven oxidative stress

Inflammatory conditions, through the activation of immune cells (in particular polymorphonuclear neutrophils; PMNC), are known to generate high amount of ROS such as O₂⁻, OH[•], which are implicated in the extensive tissue injury observed mainly in inflammatory lung disease such as COPD, cystic fibrosis, adult respiratory distress syndrome (ARDS). In turn, ROS may also exert signaling functions and regulate the transcription of inflammatory mediators, and directly induce cytokine synthesis [9]. Recently, it has been demonstrated that inflammation-induced oxidative stress seems to exert also a central role in the pathogenesis of metabolic changes responsible for cancer cachexia, cancer-related anemia, as well as AIDS. In this view, a key role is played by pro-inflammatory cytokines and ROS, which are able potently to activate intracellular pathways, i.e., nuclear factor κ B (NF- κ B) and other signals, which characterize the evolution of several chronic inflammatory diseases and are responsible for the various associated symptoms. In particular, it has been demonstrated that the glutathione depletion is directly and mainly associated with all the above-reported events [10].

3.3 The glutathione cycle

Glutathione is the most important cellular antioxidant. It is endogenously synthesized all throughout the body and it exerts several essential functions such as antioxidant defense,

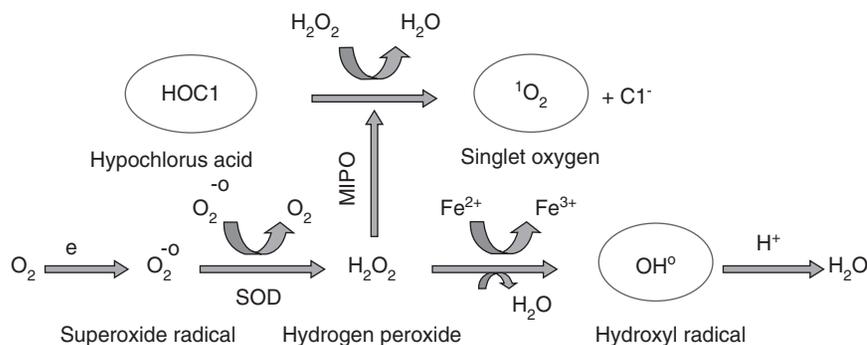


Figure 1. Generation of reactive oxygen species during the complete reduction of oxygen.

detoxification of xenobiotics, modulation of redox-regulated signal transduction, storage and transport of cysteine, regulation of cell proliferation, synthesis of deoxyribonucleotides and regulation of immune responses. Relative to glutathione availability, one of the most important issues is to maintain the blood availability of cysteine as that it is known to be the rate-limiting substrate for glutathione resynthesis. Subsequently, identifying ways to achieve an optimal availability of cysteine is a primary approach to maintain an adequate cell biosynthesis of reduced glutathione. Carbocysteine, together with *N*-acetyl cysteine (NAC) and α -lipoic acid, is one of the agents to obtain this effect.

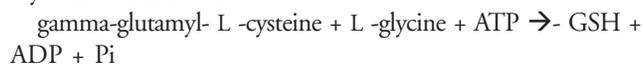
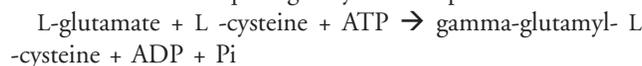
Glutathione is a tripeptide, γ -L-glutamyl-L-cysteinyl-glycine, found in all mammalian tissues and is especially highly concentrated in the liver (Figure 2). Glutathione exists in a thiol-reduced (GSH) and a disulfide-oxidized (GSSG) form [11]. GSH serves several vital functions [11-14] including: detoxifying electrophiles; scavenging free radicals; maintaining the essential thiol status of proteins; providing a reservoir for cysteine; and modulating critical cellular processes such as DNA synthesis, microtubular-related processes, and immune function. In addition, GSH has been shown to regulate nitric oxide homeostasis [15] and to modulate the activity of proteins by post-translational modification (protein S-glutathionylation) [16], neurotransmitter receptor activity [17] and the blastic response and function of T-lymphocytes.

GSH, which is the dominant non-protein thiol in mammalian cells, undergoes thiol-disulfide exchange in a reaction catalyzed by thiol-transferase as follows: Protein-S-SG + GSH \rightarrow Protein-SH + GSSG. Since this reaction is a reversible reaction, the equilibrium is determined by the redox state of the cell, which depends on the concentrations of GSH and GSSG [18].

Mitochondrial GSH is critical in defending against both physiologically and pathologically generated oxidative stress [19,20]. Severe oxidative stress can overcome the cell's ability to reduce GSSG to GSH, leading to accumulation of GSSG. To protect the cell from a shift in the redox equilibrium, GSSG can be actively exported out of the cell or react with a protein sulfhydryl group, such as cysteine, leading to the

formation of a mixed disulfide. Thus, severe oxidative stress depletes cellular GSH [21].

The synthesis of GSH from its constituent amino acids involves two ATP-requiring enzymatic steps:



The major determinants of GSH synthesis are the availability of cysteine, the sulfur amino acid precursor, and the activity of the rate-limiting enzyme, glutamate cysteine ligase (GCL). Cysteine is derived normally from the diet, protein breakdown and in the liver, from methionine via transsulfuration (conversion of homocysteine to cysteine) [18]. Through the γ -glutamyl cycle cysteine is incorporated into GSH. Storage of cysteine is one of the most important functions of GSH because cysteine is extremely unstable extracellularly and rapidly auto-oxidizes to cystine [22].

4. Carbocysteine

4.1 Chemistry and mechanism of action

Carbocysteine (R-2-amino-3[(carboxymethyl)thio] propionic acid) is a dibasic amino acid, biologically active, commonly used as a mucolytic drug. The carbocysteine molecule (Figure 3) is characterized by the presence of a bound sulfhydryl group, differently from other cysteine-derivatives, such as *N*-acetylcysteine, that contain a free sulfhydryl group [23].

Preclinical and clinical studies on pharmacological properties of carbocysteine have demonstrated that this cysteine-derivative has the ability to augment the synthesis of sialomucines, fundamental components of bronchial mucus. SCMC is converted into a number of thioether and sulfoxide metabolites including a mixed disulfide metabolite (S-(carboxymethylthio)-L-cysteine) as minor metabolites. Moreover, SCMC is decarboxylated into S-methyl-L-cysteine (SMC) but de-methylation of SMC into L-cysteine in humans is unknown.

Metabolites of cysteine include GSH, inorganic sulfur and taurine, which are essential for a wide variety of critical functions in the body (Figure 4). As widely described

Carbocysteine

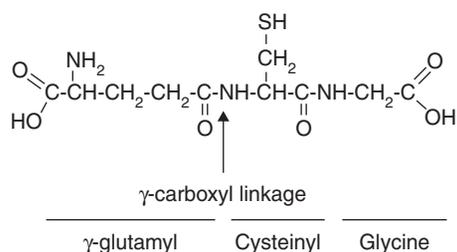


Figure 2. Chemical structure of glutathione.

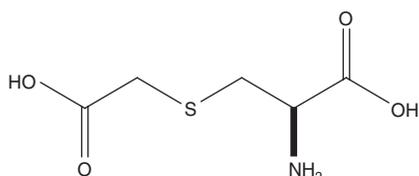


Figure 3. Chemical structure of carbocysteine.

above, GSH is involved in the maintenance of the cellular thiol-disulfide ratio, serves as a co-substrate in various enzymatic reactions and as a reservoir of cysteine. Reduced sulfur is required for the synthesis of certain macromolecules. Sulfate is required for numerous sulfation reactions, including the formation of sulfate esters of drugs as a detoxification mechanism. The role of taurine in bile acid conjugation is well known, whereas the specific role of taurine in other processes has not been clearly elucidated.

4.1.1 Carbocysteine antioxidant properties

The SCMC-lys possesses antioxidant properties in cell-free and cellular systems. In particular, SCMC-lys is a selective scavenger of HOCl and OH[•]. The capacity of SCMC-lys to scavenge OH[•] and HOCl is related to the reactivity of its thioether group which may react with ROS, oxidating itself to sulfoxide and sulfone derivatives and subsequent generation of SCMC = O. The scavenger capacity of SCMC-lys against HOCl and OH[•] was comparable to GSH, which is the main endogenous antioxidant. The scavenger effects of SCMC-lys were also observed on stimulated peripheral blood mononuclear cells (PBMCs), suggesting that SCMC-lys could exert an anti-inflammatory action by the scavenging of ROS produced by activated PBMCs [24]. Moreover, it has been reported that SCMC-lys inhibits elastase-induced conversion of xanthine dehydrogenase to xanthine-oxidase in human endothelial cells, this effect being paralleled by a significant reduction in O₂⁻ production [25]. SCMC-lys scavenger capacity on the HOCl produced by activated PBMC, is paralleled by preservation of α-IAT activity, whose inactivation by oxidative stress is implicated in the extensive tissue injury observed in pulmonary inflammatory diseases

including ARDS [26] and chronic emphysema [27]. This mechanism of action further supports how the anti-oxidant capacity of SCMC-lys could contribute to its therapeutic efficacy in chronic pulmonary diseases as well as in different chronic inflammatory diseases, such as advanced cancer and congestive heart failure.

4.1.2 Carbocysteine anti-inflammatory properties

Cysteine-containing drugs, such as SCMC-lys, and antioxidant agents together with other glutathione prodrugs were found to decrease the production of pro-inflammatory cytokines (TNF-α, IL-6, IL-8) [28]. Several *in vitro* studies showed that the antioxidant activity of SCMC-lys was paralleled by inhibition of IL-8 production. It was reported that production of IL-8 could be mediated, at least in part, by intracellular OH[•] [29]. Therefore, the ability of SCMC-lys to inhibit IL-8 production seems to be related to its scavenger capacity on OH[•] [24]. Since the chemotactic recruitment of PBMCs into the lung by IL-8 plays a crucial role in the development and maintenance of several inflammatory diseases including ARDS [30], idiopathic pulmonary fibrosis [31] and cystic fibrosis [32], the inhibition of IL-8 production could contribute to the therapeutic effect of SCMC-lys.

4.1.3 Carbocysteine mucolytic properties

Carbocysteine lysine salt monohydrate is a well-known mucoactive drug effective in acute and chronic inflammatory lung pathologies. Its mucolytic efficacy is related to its capacity to replace fucomucins by sialomucins, thereby reducing mucus viscosity [24]. Carbocysteine was able to inhibit the TNF-α-induced overexpression of sialyl-Lewis epitopes in mucins secreted by patients suffering from various respiratory diseases, such as chronic bronchitis, asthma and cystic fibrosis [33]. Furthermore, CLS increases Cl⁻ secretion in rabbit, an effect that might contribute to the overall mucoregulatory action [34].

4.2 Pharmacokinetics and metabolism

Carbocysteine is administered orally in liquid or solid dosage forms including syrup, tablet and capsule. Carbocysteine is rapidly well absorbed after oral administration and the subsequent kinetics fit a one-compartment open model [35]. Peak serum concentrations are achieved between 1 and 2 h and the plasma half-life is 1.33 h. Carbocysteine appears to penetrate into lung tissue and respiratory mucus, suggesting local action [36]. There is evidence from animal models that carbocysteine increases chloride transport across the airway epithelium and this may also contribute to its mucoregulatory action [34].

Metabolism of carbocysteine is known to be especially complex, with the pathways of decarboxylation, *N*-acetylation, sulfoxidation, deamination/transamination and ester glucuronidation all being involved to differing degrees. The majority of the drug is eliminated unchanged by urinary excretion [37]: the major urinary metabolite of SCMC was believed to be a

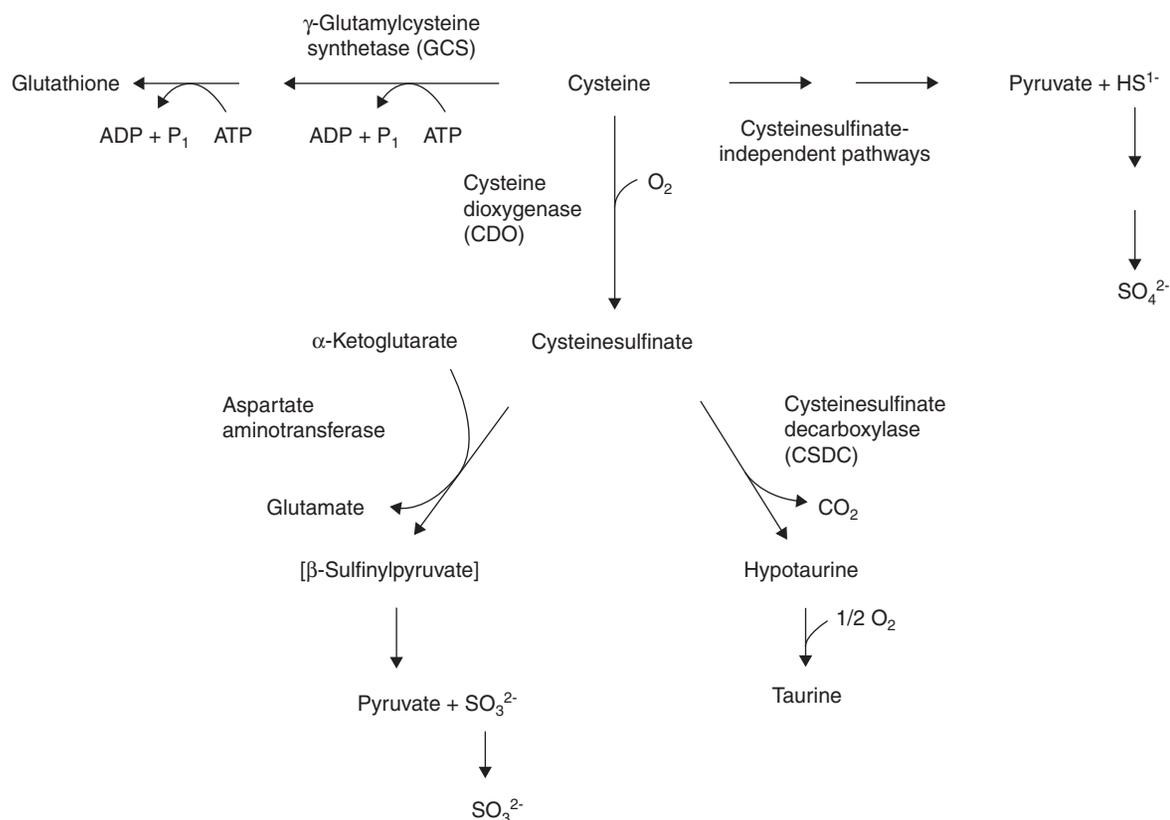


Figure 4. Metabolites of cysteine.

mixed disulfide [S-(carboxymethylthio)-L-cysteine(CMTC)] [38]. Several studies have indicated that the metabolism of carbocysteine varies widely within the same individual, with few sulfoxide metabolites being produced after nocturnal administration [39]. In particular, the timing of SCMC administration has a profound effect on the identity of urinary S-oxide metabolites produced. After daytime ingestion S-oxidation seems to be the predominate route during the first 8 h, whilst administration at night-time results initially in the formation of thiodiglycolic acid (TDA) via α -amino group deamination/transamination and subsequent decarboxylation, followed by disulfide formation and S-oxidation of TDA to form TDASO. The use of these alternative metabolic routes could possibly be due to circadian rhythm in the endocrine system, which may modulate the S-oxidation of disulfides in the mammalian body [39]. Such diurnal variation in metabolism is overlaid on an underlying and apparently genetically determined ability to produce sulfur-oxygenated metabolites. This later spread of 'sulfoxidation capacities' separates individuals with respect to their metabolic handling of the drug [40]. With these underlying differences in metabolic handling, it may be reasonable to assume that a standard therapeutic dose of carbocysteine may be more effective in some patients than others, and may even be ineffective in some. A simple shifting of dosing from

morning to night may influence efficacy. Moreover, differences in metabolism may be linked to a number of adverse drug reactions and disease states. For example, an impaired sulfoxidation of S-carboxymethylcysteine has been closely associated with primary biliary cirrhosis [41].

5. Preclinical studies

Preclinical experimental findings in rodents suggest that carbocysteine promotes the repair of damaged epithelium by allergic reaction and may be useful in allergic airway diseases accompanied by isolated chronic coughing, especially eosinophilic bronchitis without asthma and tracheobronchitis with cough hypersensitivity [42]. Animal studies have demonstrated the anti-inflammatory action of carbocysteine in models of pulmonary inflammation involving several different cytokine profiles. Oral treatment with SCMC-lys appears to attenuate neutrophil recruitment in inflammatory lung diseases [43,23]. Administration of carbocysteine to rats with sulfur-dioxide-induced airway inflammation attenuates the secretion of abnormal mucous glycoproteins and reduces inflammatory cells, free radical and elastase activity [44]. Another experimental study on cultured lines of epithelial respiratory cells demonstrated that SCMC-lys is able to stimulate a channel-mediated GSH secretion by human

respiratory cells: this mechanism of action is of great interest since GSH is one of the most important defense mechanisms against oxidative stress in the respiratory epithelial lining fluid [45].

6. Clinical efficacy

6.1 Carbocysteine in chronic obstructive pulmonary disease (COPD)

COPD is defined as 'a disease state characterized by airflow limitation that is not fully reversible but progressive, and associated with an abnormal inflammatory response of the lungs to noxious particles or gases' [46]. COPD pathophysiology involves many components including mucus hypersecretion, oxidative stress and inflammation in the airway and lungs. Therefore, agents active with mucolytic, anti-inflammatory and antioxidant effects could offer the best promise for treatment [47].

Notwithstanding the key role of mucus in aggravating airflow obstruction, the value of mucolytic therapy has been for a long time unrecognized to the point that the European Respiratory Society and the American Thoracic Society COPD guidelines discouraged the use of mucolytics in the treatment of patients with stable and/or acute COPD [48-50]. Of late, the point of view of researchers and clinicians in this regard seems to be starting to change in as much as several thiol-containing mucolytic drugs have been shown to be associated with their therapeutic effect, anti-inflammatory and antioxidant capabilities [51,52]. The efficacy of mucolytics, in particular NAC, to improve clinical and biological outcomes in patients with COPD seems to be better with increasing doses: a study by Zuin *et al.* showed that NAC 1200 mg/day was more efficacious than NAC 600 mg/day in reducing IL-8 levels and difficulty of expectoration [53].

In Europe and Asia, mucolytics such as carbocysteine have been widely used in the treatment of respiratory diseases with phlegm production because of their capacity to facilitate sputum elimination. Additionally, clinical studies have shown benefits in preventing exacerbation of COPD with carbocysteine. In 1996, a multicenter double-blind, placebo-controlled trial carried out on 662 outpatients with COPD demonstrated that continuous administration of SCMC-lys 2.7 g/day throughout the winter season was effective in reducing the incidence of exacerbations, the average number of days with acute respiratory illness, and the associated consumption of antibiotics [54]. Consistently with these results, a more recent prospective, randomized, double-blind, controlled trial in patients with COPD showed that the use of carbocysteine 1500 mg/day was closely associated with a lower frequency of common colds and exacerbations compared with placebo [54]. The ability of carbocysteine to reduce viral [55] and bacterial [56] adherence in respiratory airway epithelial cells might be one of the mechanisms by which it seems to reduce recurrent exacerbations of COPD.

Accordingly, a systematic review including 26 randomized, placebo-controlled studies recruiting 7335 participants with COPD showed that treatment with mucolytics, including carbocysteine for at least 2 months, were effective in reducing the number of exacerbations and improving health status in all studies except in the BRONCUS study, which used *N*-acetylcysteine [57]. However, since evidence on long-term efficacy was insufficient, mucolytics were not recommended for regular treatment by guidelines such as that of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) [3].

New insights into COPD care have arisen from the recent results of the PEACE trial. PEACE was a large, randomized, double-blind, placebo-controlled, parallel-group study of 709 patients which showed that long-term use of carbocysteine (1500 mg/day for 1 year) reduced the rate of exacerbations of COPD. The advantage of carbocysteine over placebo in prevention of an exacerbation was noteworthy, even after adjustment for COPD severity and concomitant therapy. Moreover, the authors did not find any difference in exacerbation rate between the carbocysteine group and the placebo group at early treatment (3 months), whilst the preventive effects were found in patients treated with carbocysteine for 6 months or more, suggesting that the longer the carbocysteine administration, the better the preventive effects on recurrent exacerbation.

Moreover, the PEACE study showed that there were significantly fewer patients with more than one exacerbation in the carbocysteine group than in the placebo group. These results are reinforced by additional analyses carried out by the authors on the probability of recurrent exacerbation (more than one exacerbation) with the log-rank test: they showed that recurrent exacerbation was less likely to occur in the carbocysteine group than in the placebo group [58].

There were no interactions between treatment and COPD severity, smoking status, and concomitant use of inhaled corticosteroids differently from the BRONCUS study, which demonstrated a significant reduction in exacerbation rate by *N*-acetylcysteine only in patients without concomitant use of inhaled corticosteroids [59]. In addition to preventing COPD exacerbations, carbocysteine was shown to improve the patients' quality of life. After 1 year of treatment with carbocysteine, significant improvements over placebo were achieved in the quality of life questionnaire used (i.e., St George's Respiratory Questionnaire) both in total and symptom scores. The activity score also seemed favorable with carbocysteine versus placebo.

According to these results, the antioxidant and anti-inflammatory properties of carbocysteine seem to be important for the therapeutic efficacy of this drug. Indeed, since a variety of oxidants and free radicals are implicated in the pathogenesis of COPD, it is possible that therapeutic administration of multiple antioxidants (i.e., glutathione, *N*-acetyl cysteine, carbocysteine lysine salt, pholiphenols) may be effective in the treatment of COPD [60].

So far the efficacy of carbocysteine has not yet been tested in comparison with that of standard drugs, i.e., inhaled corticosteroids or broncodilators, in randomized trials. Therefore, even if inhaled corticosteroids, long-acting β_2 agonists and anti-cholinergics would be preferable for better outcomes, mucolytics such as carbocysteine might represent an important alternative where corticosteroid use is contraindicated, and might have a role in the treatment of COPD, particularly for long-term use.

Therefore, according to this evidence, mucolytics such as carbocysteine should be recognized as a worthwhile treatment for the long-term management of COPD.

6.2 Carbocysteine in cancer-related anorexia/cachexia syndrome

The progression of neoplastic disease is characterized by specific alterations of energy metabolism and by symptoms like fatigue, anorexia, nausea, anemia and immunodepression which finally result in a peculiar clinical picture known as cancer cachexia syndrome (CACS), which, unless counteracted, can lead to a patient's death. Both the tumor growth and the host immune aspecific activation are responsible for these processes with progressive weight loss and worsening of patient performance status (PS). In advanced cancer patients, the altered energy metabolism, in addition to symptoms such as anorexia, nausea and vomiting, do not allow an adequate synthesis of reducing compounds and a normal intake of carbohydrates and dietary antioxidants, thus favoring the accumulation of ROS. Therefore, in advanced cancer patients oxidative stress may be considered a manifestation of reduced food intake and impaired glucose utilization [61].

In a series of our recently published studies [7,62,63], we have demonstrated that patients with advanced-stage cancer showed a condition of oxidative stress characterized by high blood concentrations of ROS and reduced erythrocyte glutathione peroxidase and superoxide dismutase activity. Moreover, oxidative stress was associated with high concentrations of proinflammatory cytokines IL-6 and TNF- α , and CPR, and low levels of leptin.

In particular, the inverse correlation between leptin levels and the parameters of oxidative stress (OS) strongly suggest that leptin is a signal of negative energy balance and low energy reserves and that oxidative stress is the consequence of the metabolic derangement, particularly of glucose metabolism. Therefore, OS, consequent in advanced cancer patients to the low energy reserves and the inability to utilize efficiently the energy substrates, particularly glucose, may be considered the direct evidence of the metabolic impairment of which leptin is the most important parameter. Accordingly, in a recent paper [61] we demonstrated that in advanced ovarian cancer patients the lowest leptin levels and the highest IL-6 levels correlated with the highest levels of ROS and the lowest levels of GPx.

Moreover, the impairment of energy metabolism, by inducing oxidative stress, is responsible for defective immune functions

shown in advanced cancer patients. Immunodepression is a key feature of patients with CACS, and its severity is related to the stage of disease and severity of cachexia. Several of our studies demonstrated that PBMCs isolated from cancer patients show an impaired blastic response to mitogens (such as PHA, anti-CD3 antibody and recombinant IL-2). These defective functions correlate with the severity of disease and poor survival and are actually considered a consequence of the oxidative stress, which in turn is an effect of the cell's impaired glucose metabolism. In advanced cancer patients, the altered energy metabolism and particularly the defective glucose utilization are responsible for the reduced synthesis of reducing compounds by the pentose – phosphate pathways. However, the correct immune cell functioning requires adequate concentrations of intracellular reducing compounds and particularly GSH. Several studies have widely demonstrated that the supplementation of GSH to the medium of cultured T-cells increases the IL-2 receptor expression, as well as its internalization and degradation, and ameliorates the blastic response of lymphocytes to PHA, anti-CD3 and recombinant IL-2. These hypotheses have been confirmed by several of our *in vitro* experiments [64,65], which demonstrated that, by adding different thiol-containing antioxidants to the medium of cultured PBMCs isolated from advanced cancer patients, we were able to reverse *in vitro* the most significant functional defects of immune cells, such as response to mitogen (PHA) and antigens (anti-CD3), the expression of surface activation markers (CD25 and CD95) and cell cycle progression (from G1 to S phase).

Therefore, the correction of oxidative stress by appropriate antioxidants may reverse immunological deficits *in vivo*. In our study [66], we demonstrated that, in a population of advanced cancer patients with tumor at different sites, different cysteine-containing compounds (including carbocysteine lysine salt 2.7 g/day orally) administered alone or in combination were effective in reducing ROS and increasing GPx activity. The antioxidant treatment also reduced serum levels of IL-6 and TNF- α .

On the basis of this rationale, we have introduced the cysteine-derivative compound, carbocysteine lysine salt, in a combined dietary, nutritional and pharmacologic approach with the aim to normalize the metabolic environment associated with cancer cachexia and to improve the associated symptoms that affect quality of life. Indeed, in a first Phase II non-randomized study, we demonstrated the efficacy of a combination regimen including carbocysteine lysine salt 2.7 g/day in a population of advanced cancer patients with tumors at different sites with cachexia. The combination regimen was administered continuously for 4 months and demonstrated to be effective in ameliorating lean body mass, fatigue and blood concentrations of pro-inflammatory cytokines [67,68]. More recently, in a randomized Phase III study aimed to test the safety and efficacy of different treatment arms on cancer-related cachexia,

the administration of carbocysteine has been demonstrated to be safe and effective in combination to different anti-cachectic drugs (progestagens, L-carnitine, thalidomide) in ameliorating some main symptoms of cancer cachexia [69].

However, these positive results in improving oxidative stress and some key parameters of cancer cachexia have been obtained by supplementing patients with carbocysteine in the context of a combination regimen. Therefore, the specific role of carbocysteine in mediating these beneficial effects remains undetermined. However, it is to be highlighted that there is evidence, recognized by leading experts in this field [70], that cachexia as a multifactorial disease with a complex pathogenesis requires a combined multitargeted approach for its effective treatment.

6.3 New prospectives: cysteine-containing drugs in cardiovascular and neurodegenerative diseases

6.3.1 Cardiovascular diseases

The heart is one of the most prominent oxygen-consuming organs, and oxidative stress is associated with most of the cardiovascular diseases and heart failures (e.g., chronic heart failure). The molar ratio of free radicals increased directly after cardiopulmonary bypass surgery. Myocardial infarction after ischemia-reperfusion is a consequence not only of necrosis but also apoptosis, and ROS are most probably the signaling molecules. Therefore, to prevent ROS-induced disorders, the heart needs effective antioxidant systems [71]. Over the years a number of thiol-containing compounds have been used experimentally to inhibit LDL oxidation and reduce oxidative stress [72]. Recent studies would support the continued investigation of such compounds. In glucose-fed rats, α -lipoic acid attenuated hypertension, insulin resistance and oxidative stress and in another study was shown to reduce blood pressure in spontaneously hypertensive rats [73]. In humans, the classical sulfhydryl compound *N*-acetylcysteine reduced cardiovascular events in patients with end-stage renal failure [74].

6.3.2 Neurodegenerative disorders

The concept that oxidative stress contributes to the pathogenesis of neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease and the inherited ataxias, has prompted attempts to treat these conditions with various antioxidants [75].

7. Safety and tolerability

In a recent systematic review of randomized controlled trials on the use of oral mucolytic drugs for exacerbations of COPD, Poole reported that mucolytic-associated adverse events were usually mild and self-limiting [57]. In the above-reported PEACE multicentre, randomized, double-blind, placebo-controlled study, the most common adverse events reported were gastrointestinal problems (14 cases in the carbocysteine group vs 5 cases in the placebo group) [47].

Together with gastrointestinal disturbances, dermatological reactions are among the few side-effects associated with SCMC therapy. Ten cases of SCMC-induced fixed drug eruption have been observed only in Japanese individuals [76]. The duration between the initial intake of SCMC and the development of skin symptoms varied from 2 to 4 days. Thiodiglycolic acid (TDA), a metabolite of SCMC, has been suggested to cause the fixed drug eruption observed in two Japanese females following SCMC administration. However, the carboxymethiol metabolite should also be considered as the prime causative agent. This metabolite is highly reactive, unlike the TDA itself. The free thiol group in this metabolite avidly forms disulfide compounds with other low-molecular-weight thiols (e.g., cysteine, glutathione), thereby removing protective agents, and also readily interacts with the sulfhydryl groups within proteins and other macromolecules. These latter combinations may well precipitate allergic reactions within susceptible individuals [77]. Moreover, genetic predisposition may play an important role in the induction of SCMS-induced fixed drug eruption [39].

A case of drug-induced pneumonia has been observed in a 70-year-old during L-carbocysteine treatment for the common cold. Symptoms and laboratory findings improved markedly after stopping L-carbocysteine administration and starting corticosteroid therapy. To our knowledge, this is possibly the first case of L-carbocysteine-induced pneumonia to be reported [78].

8. Conclusions

Carbocysteine has been recently recognized as an effective and safe treatment for the long-term management of COPD, able to reduce the incidence of exacerbations and improve patient quality of life. Its efficacy in treating COPD exacerbations seems to be superior to the other mucolytics tested so far. Moreover, carbocysteine has been shown to improve oxidative stress and chronic inflammation associated with severe chronic diseases, in particular advanced cancer and cancer-related anorexia/cachexia syndrome, both alone and in combination with other antioxidant drugs. Further controlled trials are required to compare better its efficacy compared with other similar drugs (i.e., *N*-acetyl cysteine) and to assess the best dosage and modality of combination with other drugs.

9. Expert opinion

Supplementation with antioxidants, either through increased consumption in the diet or from supplementation, has become extremely popular as a mean to improve one's health or increase physical performance. It has been suggested that increasing the circulating levels of certain antioxidants (e.g., glutathione, *N*-acetyl-cysteine, α -lipoic acid, vitamins A, E, C, etc.) will help to prevent the accumulation of free radicals inside our cells, thus reducing oxidative stress. By

decreasing oxidative stress, researchers have suggested that the risk and the progression of several inflammatory chronic diseases (i.e., cancer, Parkinson's disease, Alzheimer's disease) may all be decreased. However, this approach based on supplementation with antioxidants has failed in some clinical trials aiming at the prevention of degenerative diseases and in studies involving patients with acute and chronic disease [79-83].

Several experimental and clinical studies have demonstrated that the antioxidant and anti-inflammatory properties of carbocysteine are more important than mucolysis itself for the therapeutic efficacy of this drug. Carbocysteine is able effectively to counteract the oxidative stress and the inflammatory pathways, and these therapeutic targets may be relevant also for its impact on patient quality of life. Indeed, carbocysteine has been used effectively for its anti-inflammatory and antioxidant capacities, in the restoration of the immune deficits and the cachectic symptoms associated with advanced cancer.

Moreover, it is to be underlined that the metabolism of carbocysteine is particularly complex and may be widely

different both within the same individual according to a diurnal variation and in different patients due to genetically determined metabolic capacities. Consequently, a standard therapeutic dose of carbocysteine may be more effective in some patients than in others and may even be ineffective in some. Therefore, further clinical studies to assess better the different efficacy of carbocysteine for specific groups of patients are warranted.

Therefore, beyond its well-demonstrated, but limited, clinical applications, carbocysteine, as well as the other thiol-containing drugs (i.e., *N*-acetylcysteine) may be useful in the management and treatment of several chronic inflammatory diseases, such as cardiovascular diseases and neurodegenerative disorders. Controlled, randomized studies are warranted.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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