Targeting oxidant-dependent mechanisms for the treatment of COPD and its comorbidities

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Abstract

Chronic obstructive pulmonary disease (COPD) is an incurable global health burden and is characterised by progressive airflow limitation and loss of lung function. In addition to the pulmonary impact of the disease, COPD patients often develop comorbid diseases such as cardiovascular disease, skeletal muscle wasting, lung cancer and osteoporosis. One key feature of COPD, yet often underappreciated, is the contribution of oxidative stress in the onset and development of the disease. Patients experience an increased burden of oxidative stress due to the combined effects of excess reactive oxygen species (ROS) and nitrogen species (RNS) generation, antioxidant depletion and reduced antioxidant enzyme activity. Currently, there is a lack of effective treatments for COPD, and an even greater lack of research regarding interventions that treat both COPD and its comorbidities. Due to the involvement of oxidative stress in the pathogenesis of COPD and many of its comorbidities, a unique therapeutic opportunity arises where the treatment of a multitude of diseases may be possible with only one therapeutic target. In this review, oxidative stress and the roles of ROS/RNS in the context of COPD and comorbid cardiovascular disease, skeletal muscle wasting, lung cancer, and osteoporosis are discussed and the potential for therapeutic benefit of anti-oxidative treatment in these conditions is outlined. Because of the unique interplay between oxidative stress and these diseases, oxidative stress represents a novel target for the treatment of COPD and its comorbidities.

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1. Introduction

An increased burden of oxidative stress is an important feature of the pathogenesis of chronic obstructive pulmonary disease (COPD) and its associated comorbid diseases (comorbidities). Current forms of therapy for COPD are largely ineffective and the development of effective treatments for COPD has been severely hampered by the mechanisms and mediators that drive the induction and progression of chronic inflammation, emphysema, altered lung function, defective lung immunity and many extrapulmonary comorbidities are still poorly understood. What is known is that four primary mechanisms have been implicated in the pathophysiological alterations observed in COPD: oxidative stress; inflammation; protease–antiprotease imbalance; and apoptosis (Barnes, 2013, 2014; Hillas, Nikolakopoulou, Hussain, & Vassilakopoulos, 2013). Although mostly underappreciated, oxidative stress has been recognized as a central component in the pathogenesis of COPD as it can trigger and further potentiate the other three mechanisms. In this review we focus on the role of oxidative stress in the pathogenesis of COPD and its comorbidities. Given its pivotal role in the onset and development of COPD, oxidative stress may be a novel target for the treatment of COPD and its comorbidities.

1.1. Chronic obstructive pulmonary disease overview

COPD represents an increasing global burden, afflicting over 600 million people and corresponding to approximately 5% of all deaths globally (Koul, 2013). It is currently the third leading cause of death (Lozano et al., 2012). It is well known that lower/middle income countries bear most of the burden of COPD with almost 50% of COPD-related deaths worldwide taking place in these countries (Lopez et al., 2006). Although the incidence rate of COPD is likely to escalate in both developed and developing countries, COPD poses a heavier burden on the Asia-Pacific and African regions where smoking is still widespread and gradually increasing (Chan-Yeung, Ait-Khaled, White, Ip, & Tan, 2004; Adelayo et al., 2015). Cigarette smoke is inarguably the biggest risk factor for COPD, with 90% of deaths from COPD directly attributable to smoking (Tashkin & Murray, 2009). Other risk factors include exposure to air pollutants and biomass fuels (Song, Christiani, XiaorongWang, & Ren, 2014). In addition, there is generally a long latency period between exposure to smoke and clinically-evident disease and as such, there is a high incidence of COPD in the older population and ex-smokers (Theander et al., 2014). This latency period generally lasts a number of years and by the time patients become symptomatic, the damage is already irreversible.

COPD is characterized by persistent airflow limitation and lung inflammation resulting in a progressive decline in lung function (Angelis et al., 2014). The main symptoms of the disease are chronic cough (smoker’s cough), excessive mucus production and dyspnoea (particularly during exercise) (Theander et al., 2014). In addition to the pulmonary manifestation of COPD, many systemic manifestations occur in the form of comorbid diseases, such as skeletal muscle wasting, cardiac dysfunction, osteoporosis and lung cancer (Chatila, Thomashow, Minai, Criner, & Make, 2008; Barnes & Celli, 2009; Patel & Hurst, 2011). These comorbidities have been associated with increased oxidative stress and are known to affect, or are strong predictors of, the mortality of COPD patients independent of the decline in lung function (Schols, Slagen, Volovics, & Wouters, 1998; Marquis et al., 2002; Sin, Anthonisen, Soriano, & Agusti, 2006; Swallow et al., 2007).

Although COPD is a largely preventable disease; the numbers of diagnoses continue to increase resulting in ever increasing medical costs to patients, communities and governments (Lozano et al., 2012; GOLD, 2015). COPD patients generally have long hospital stays, require long-term treatments, and in addition to these medical costs, other “costs” such as an increase in days missed from work and limitations to quality of life, are important consequences of this disease (Rabe, 2007; Vestbo et al., 2013).

1.2. Acute exacerbations of chronic obstructive pulmonary disease

Patients with COPD often experience episodes of sudden worsening of symptoms, known as acute exacerbations (AECOPD). The Global initiative for chronic Obstructive Lung Disease (GOLD) defines an exacerbation as a “change in the patient’s baseline dyspnoea, cough, and/or sputum and beyond normal day-to-day variations, that is acute in onset and may warrant a change in regular medication in a patient with underlying COPD”. These exacerbations are considered to be part of the natural progression and chronicity of the disease and as COPD progresses, exacerbations become increasingly more frequent (Hurst & Wedzicha, 2009; Mackay & Hurst, 2013). Exacerbations result in a dramatic increase in lung inflammation and are associated with increased systemic inflammation compared to stable disease and as a result, there is only an increase in oxidative stress markers (Stanajkovic et al., 2011). This contributes to the worsening of symptoms and it has been shown that following hospitalisation for AECOPD, patients have increased mortality rates (Steer, Gibson, & Bourke, 2010).

AECOPD is often triggered by respiratory infections, such as those caused by the bacteria Streptococcus pneumoniae and Influenza A virus, or acute exposure to airborne irritants (Repine, Bast, & Lankhorst, 1997; Mackay & Hurst, 2013). There are also many non-aetiological risk factors that contribute to the frequency of exacerbations such as age, frequent past exacerbations and the presence of comorbid diseases (especially cardiovascular disease) (Laratta & van Eeden, 2014). The rates of exacerbations vary dramatically depending on the parameters used to define an exacerbation, however, rates of severe AECOPD measured by hospitalisations occur at an approximate rate of 0.5 to 3.6/person-year depending on the study cited (de Melo, Ernst, & Suisan, 2004; Seemungal, Hurst, & Wedzicha, 2009). The outcomes of AECOPD can vary from the return to near baseline spirometric parameters to respiratory failure and death. Exacerbations are the largest direct cost for the treatment of COPD due to the length of hospital stays and the frequency of the exacerbations per patient (Mirtalavits, Murio, Guerrero, Gisbert, & EPOC, 2002; Miravitlles et al., 2004; Mackay & Hurst, 2013).

2. Oxidative stress in chronic obstructive pulmonary disease

2.1. What is oxidative stress?

Oxidative stress refers to the imbalance between the oxidant and antioxidant levels in favour of a pro-oxidant environment in cells and tissues (Kalyanaraman, 2013). An oxidant is a species that causes or promotes oxidation and an antioxidant is a molecule that inhibits either the formation of oxidants or inhibits oxidation itself. Oxidative stress arises from the inability of innate antioxidant mechanisms to neutralize oxidants generated endo- or exogenously resulting in an imbalance between oxidant and antioxidant factors. Consequently, the oxidants predominate and chronic oxidative stress occurs, leading to the modification of lipids, proteins, and DNA (Rahman, 2005; Biswas, Hwang, Kirkham, & Rahman, 2013). The harmful modifications caused by oxidative stress are referred to as oxidative damage.

Oxidative stress can result from increased production of oxidants (in the form of free radicals/reactive oxygen and nitrogen species) or from diminished antioxidant levels or reduced antioxidant enzyme activity (Kalyanaraman, 2013). The depletion of dietary antioxidants (e.g., vitamins E, C, and D, flavonoids and carotenoids) and micronutrients (e.g., iron, copper, zinc; selenium) can also contribute to oxidative stress as they are needed for proper functioning of antioxidant enzymes (Delles, Xiong, True, Ao, & Dawson, 2014). In chronic inflammatory conditions, such as COPD, oxidative stress primarily results from the increased production of reactive oxygen species (ROS) from exposure to toxins (e.g., cigarette smoke, infection) and continuous activation of endogenous enzymes (e.g., NADPH oxidases) (Fig. 1).
2.1.1. Chronic obstructive pulmonary disease and oxidative stress

The prolonged increase in oxidative stress is a major factor in potentiating both the airway and systemic inflammation in COPD and is known to play a key role in the onset and development of COPD and its comorbidities (Montuschi et al., 2000; Cavailles et al., 2013; Kirkham & Barnes, 2013). Direct damage occurs due to the oxidants found in cigarette smoke and from excessive levels of ROS and reactive nitrogen species (RNS) produced as a result of both pulmonary and systemic inflammation. An increase in ROS production in the airways is reflected by elevated levels of markers of oxidative stress (e.g., superoxide and malondialdehyde) in the airspaces, sputum, breath, lungs, and blood in patients with COPD (Rahman, 2005; Stanojkovic et al., 2011). These levels of oxidative stress markers are also dramatically increased during exacerbations of COPD (Antus, Harnasi, Drozdovszky, & Barta, 2014). Due to the nature of the oxidative burden and its consequences in the progression of COPD, this review is primarily focused on the role of ROS/RNS in the pathogenesis of COPD and its comorbidities, and the potential pharmacological targets related to ROS signalling.

2.1.2. Environmentally-derived reactive oxygen species

Each puff of cigarette smoke contains more than $10^{15} - 17$ oxidant-free radical molecules and over 4700 highly reactive chemical compounds, such as aldehydes and quinones, which increases the oxidant burden in smokers (Church & Pryor, 1985; Nakayama, Church, & Pryor, 1989; Pryor & Stone, 1993; Rahman, 2005, 2012; Kirkham & Barnes, 2013). The nature of ROS found within cigarette smoke varies from short-lived oxidants, such as the superoxide radical ($O_2^{-}$) and the nitric oxide radical (NO•), to long-lived organic radicals, such as semiquinones that can undergo redox cycling within the epithelial lining fluid of smokers for some considerable period of time (Nakayama et al., 1989; Valavanidis, Vlachogianni, & Fiotakis, 2009a, 2009b). Lung and systemic formation of protein carbonyls in response to cigarette smoke-derived lipid peroxides/carbonyls have also been implicated in the pathogenesis of COPD (Montuschi et al., 2000).

In addition to the release of oxidants, cigarette smoking is also associated with an increased amount of myeloperoxidase (MPO) in neutrophils, an oxidising factor that forms hypochlorous acid and converts tyrosine to tyrosyl radical (Bridges, Fu, & Rehm, 1985). Studies have shown a correlation between the content of MPO in neutrophils and the degree of pulmonary dysfunction observed in patients (Vaguliene, Zemaitis, Lavinskiene, Miliauskas, & Sakalauskas, 2013). In addition, studies suggest that neutrophil MPO-mediated oxidative stress plays a role in lung inflammation (Gernez, Tirouvanziam, & Chanez, 2010). It is also known that cigarette smoke can cause the activation of alveolar macrophages, which is observed in the bronchoalveolar lavage fluid (BALF) from the lungs of smokers and COPD patients but not present in non-smokers. The activation of macrophages contributes to the endogenous generation of ROS in the respiratory tract (Kirkham, Spooner, Flouikes-Jones, & Calvez, 2003; Barnes, 2004).

2.1.3. Cellular derived reactive oxygen species

Cellular-derived ROS is enzymatically produced by inflammatory and epithelial cells within the lung and/or systemically as part of an inflammatory-immune response towards a pathogen or irritant (Kim et al., 2013). Production of ROS by phagocytes can be enhanced by oxidants present in cigarette smoke leading to the release of inflammatory mediators (Rahman, 2012). Several sources for ROS production exist within a cell; however, the primary ROS generator is the enzyme NADPH oxidase (NOX), which comes in various isoforms: NOX-1, NOX-2, and NOX-4 (Selemidis, Sobey, Wingler, Schmidt, & Drummond, 2008; Drummond, Selemidis, Griendling, & Sobey, 2011).
In humans, NOX-1 and -2 are significant ROS-generators which are made up of an enzyme complex which is present in phagocytic and non-phagocytic cells including epithelial cells, macrophages, and skeletal muscles (Griffith et al., 2009; Barbieri & Sestili, 2012). Although NOX is found in many cell types, it is latent in neutrophils under normal circumstances (MacNee & Rahman, 2001). Once activated, neutrophils and macrophages can generate ROS via the NADPH oxidase system, leading to further augmentation of oxidative stress in the lungs of smokers and COPD patients (Fig. 2).

NOX-generated ROS have long been recognized to play key roles in the pathogenesis of a number of diverse chronic lung disorders that result in obstructive physiology, in particular asthma, cystic fibrosis, and emphysema (Griffith et al., 2009). Mice deficient in p47phox or NOX-2 exhibit increased cigarette smoke-induced lung inflammation and emphysema despite decreased ROS production compared with control mice (Yao et al., 2008). The lung responses in p47phox- and NOX2-null mice were associated with increased production of pro-inflammatory cytokines and chemokines via a TLR4–NF-κB pathway, indicating that NOX-2 may mediate anti-inflammatory functions by restraining TLR4 activation (Yao et al., 2008). However, another group reported that p47phox-null mice have less inflammation, IL-6, keratinocyte-derived chemokine, and monocyte chemoattractant protein-1 in lung-lavage specimens after cigarette-smoke exposure compared with WT mice (Gicquel et al., 2008). The differences observed by these groups may be due to variability in lung compartment sampling, cellular distributions, and chronicity of cigarette-smoke exposure.

2.1.4. Reactive nitrogen species

Although increased production of ROS is the primary mechanism of oxidative stress in COPD/chronic lung diseases/acute lung diseases, there is compelling evidence to suggest that RNS also play a role in COPD (Ichinose, Sugiura, Yamagata, Koarai, & Shirato, 2000; Ichinose et al., 2003). Reactive nitrogen species include nitric oxide (NO•), a nitrogen free radical, and its derivative species such as peroxynitrite and nitrogen dioxide (Fig. 3). As with ROS, in addition to generation endogenously, RNS are present in cigarette smoke and air pollutants in the form of NO• and has many of the same harmful effects as ROS (Hasnis, Bar-Shai, Burbea, & Reznick, 2007). Endogenously, NO• is associated with a multitude of signalling pathways in mammalian physiological and pathological processes; however, in excess it too causes

![Fig. 2. Cellular generation of reactive oxygen and nitrogen species in COPD. Cigarette smoke acts on inflammatory cells in the lung (e.g., macrophages, neutrophils, epithelium) where activation of NADPH oxidase 2 (Nox2) generates superoxide radicals. (O2−•) which can then either react with nitric oxide (NO•) to form the reactive peroxynitrite molecule (ONOO•−) or be rapidly converted to hydrogen peroxide (H2O2) via the enzymatic activity of superoxide dismutase (SOD). In the presence of Fe2+, H2O2 can be converted into the more damaging hydroxyl radical (•OH) via the Fenton reaction. This reaction causes the oxidation of Fe2+ to Fe3+, and in this oxidation state, the presence of iron can directly generate •OH from O2−•. These iron reactions have increased importance in COPD as a higher concentration of iron has been reported in the lungs of smokers, thereby increasing the potential ROS burden (Gloire et al., 2006). The glutathione peroxidase (Gpx) family of enzymes, and catalase (CAT) are responsible for the conversion of H2O2 into harmless water and oxygen, which effectively reduces circulating ROS and thus reduces the oxidative burden (Vlahos & Bozinovski, 2013).](image)

![Fig. 3. The reactive nitrogen species cascade. In addition to its ability to cause direct damage, ONOO•− can also react with carbon dioxide (CO2) in vivo. The reaction between ONOO•− and CO2 occurs rapidly under physiological conditions, forming nitrosoperoxycarbonate (ONOOCO2−). ONOOCO2− homolyzes (the molecule dissociates into 2 free radicals) to form the carbonate radical (•CO32−) and nitrogen dioxide (•NO2). (Kalyanaraman, 2013). It is these radicals (•CO32− and •NO2) that are believed to cause peroxynitrite-related cellular damage. The conjugate acid of peroxynitrite, peroxynitrous acid (HNO3) also homolyzes into •OH and •NO2 radicals, adding to the oxidative burden caused by peroxynitrite (Merényi, Lind, Goldstein, & Czapski, 1998).](image)
indiscriminate damage to surrounding tissues and can react with O$_2^*$- forming the even more harmful peroxynitrite radical (ONOO$^-$).

2.1.4.1. Nitric oxide. For years, NO has only been considered as a toxic, unstable free radical gas that was just a constituent of air pollutant and cigarette smoke. However, it is now known that NO can be generated endogenously in several types of cells (Bredt, 1999). This implicated NO in various physiological roles and pathways, including host defence, vascular regulation and neuronal communication (Kalyanaraman, 2013). NO has been well characterized in human biology and is perhaps the most important endogenous vasoprotective molecule in addition to its role in cardiovascular function (Moncada & Higgs, 2006; Tang & Vanhoutte, 2009; Vanhoutte, Shimokawa, Tang, & Feletou, 2009; Feletou, Kohler, & Vanhoutte, 2012). NO inhibits vascular smooth muscle contraction and growth, platelet aggregation, and leukocyte adhesion to the endothelium, contributing to vascular homeostasis (Cooke, 2004; Moncada & Higgs, 2006; Tang & Vanhoutte, 2009; Vanhoutte et al., 2009; Feletou et al., 2012; Trigg et al., 2012). Individuals with hypertension, atherosclerosis and/or diabetes often show impaired NO signalling, highlighting the importance of NO with regards to comorbid cardiovascular disease (Huang, 2009) (see below).

Nitric oxide is generated by phagocytes and is biosynthesized endogenously from the amino acid L-arginine, oxygen, and NADPH by various nitric oxide synthase (NOS) enzymes (Palmer, Ashton, & Moncada, 1988). In addition to its signalling roles, NO has both pro-oxidant and antioxidant activities; excessive NO can cause direct oxidative damage, however, NO can also scavenge circulating ROS (Joshi, Ponthier, & Lancaster, 1999; Wink et al., 2001). More importantly, O$_2^*$- can react directly with endothelium-derived NO forming the harmful ONOO$^-$ molecule.

2.1.4.2. Peroxynitrite. Peroxynitrite is formed by the reaction between O$_2^*$- and NO in vivo. The pairing of these 2 radicals results in ONOO$^-$ anion, which is not a free radical but is still a very potent oxidant. ONOO$^-$ is both an oxidising and nitrating agent and can thus damage a wide array of molecules, including DNA and proteins (Beckman, 1996; Szabo & Olshina, 1997). The formation of ONOO$^-$ is of particular relevance to the comorbidities of COPD as its formation involves the consumption of NO, reducing the bioavailability of NO for physiological processes (see Section 3.2.3).

2.1.5. Depletion of antioxidants

ROS/RNS generated exogenously or endogenously, whether circulating or in pulmonary vasculature, are scavenged by blood antioxidants and antioxidant enzymes. Accordingly, the ability to protect against the deleterious effects of oxidative stress depends greatly on the antioxidant capacity of the blood and the tissues (Rahman, Morrison, Donaldson, & MacNee, 1996; Rahal et al., 2014). Studies have shown that in addition to the increased levels of circulating oxidants, there is also an observed decrease in systemic antioxidant capacity in smokers and patients with COPD (Rahman, Swarska, Henry, Stolk, & MacNee, 2000). This is due to the saturation of lung antioxidants, plasma antioxidants and antioxidant protein and sulphydryls by the excessive amounts of circulating ROS released by neutrophils and macrophages (Rahman et al., 2000). The saturation of many of these antioxidants, such as uric acid, glutathione (GSH), vitamin E, and ascorbate is also associated with the severity of COPD exacerbations (Rahman, Skwarska, & MacNee, 1997). Cigarette smoke, the main aetiological risk factor for COPD, has also been shown to irreversibly modify glutathione to glutathione conjugates in the airway epithelium resulting in antioxidant deficiency and injurious lung response (van der Toorn et al., 2007). Cigarette smoking also inhibits the protective expression of the Nr2 antagonist response element pathway in peripheral mononuclear cells of smokers, favouring a pro-inflammatory state (Garbin et al., 2008). In addition to the antioxidant saturation, studies have also shown a decrease in anti-oxidative enzyme function in COPD patients; specifically reduced SOD and Gpx activity (Kurys, Kurys, Kuzniar, & Kieszko, 2001).

There is evidence to suggest that the anti-oxidant enzyme glutathione peroxidase-1 (Gpx-1) may have a role in regulating the inflammatory response to cigarette smoke exposure. Elevated levels of H$_2$O$_2$ are measured in the exhaled breath condensate of COPD patients, particularly during exacerbations (Dekhuijzen et al., 1996). There is upregulation of Gpx-1 gene expression in the lungs of smokers (Bentley, Emrani, & Cassano, 2008) and depletion of Gpx activity in COPD patients and smokers (Santos et al., 2004; Kluchova, Petrasova, Joppa, Dorkova, & Tkacova, 2007; Vibhuti, Arif, Deepak, Singh, & Qadar Pasha, 2007). With respect to reduced Gpx activity in COPD patients and smokers, erythrocyte Gpx activity was significantly lower in patients with severe COPD compared with patients with moderate COPD and there is a direct relationship between systemic Gpx activity and FEV$_1$ (Kluchova et al., 2007). In addition, Gpx activity was decreased in plasma from COPD patients and oxidative stress correlates with both lung function and body mass index in COPD (Vibhuti et al., 2007). Moreover, Gpx activity was decreased in total blood from smokers and ex-smokers (Santos et al., 2004). However, these studies did not identify the isoform of Gpx that was involved in reduced activity of Gpx.

2.2. Oxidative modifications

Oxidative stress causes a wide array of physiological and pathological consequences not necessarily limited to just COPD patients. In COPD, increased oxidative stress can cause cell damage, cell necrosis, apoptosis, autophagy, remodelling of extracellular matrix and blood vessels, endothelial dysfunction, inactivation of antiproteases, premature cellular senescence, elevated mucus secretion, steroid resistance, unfolded protein response, cell proliferation, epigenetic changes, and autoimmunity (Rahman, 2005; Rahman & Innula, 2012; Kirkham & Barnes, 2013). Oxidative modifications of DNA, proteins and lipids all contribute to the pathophysiology of the disease.

2.2.1. DNA

Unlike the other ROS and RNS which do not react with DNA bases or deoxyribose, the hydroxyl radical and peroxynitrite can react with both purine (adenine & guanine) and pyrimidine (cytosine & thymine), forming specific products (Cooke, Evans, Dizdaroglu, & Lunc, 2003). This oxidative damage can lead to mutation of DNA bases caused by AT–GC transition and GC–AT transversion and, if left unrepaired, can result in changes in protein gene expression (Kalyanaraman, 2013). In addition to this, ROS/RNS-induced DNA damage also involves single- or double-stranded DNA breaks and DNA cross-links, DNA damage inducing either arrest or induction of transcription, induction of signal transduction pathways, replication errors, and genomic instability (Naito, Suematsu, & Yoshikawa, 2011).

2.2.2. Protein

Oxidative modifications to proteins caused by ROS/RNS include protein fragmentation, oxidation of amino acids, the formation of carbonyls, dityrosine and nitrosated and chlorinated tyrosines (Berlett & Stadtman, 1997; Grimsrud, Xie, Griffin, & Bernlohr, 2008). In COPD and other inflammatory diseases, elevated levels of nitrosated, chlorinated and brominated tyrosines have been detected in the tissues of patients (Kim, Mofarrah & Hussain, 2008). These oxidative changes to proteins can result in various functional consequences, such as inhibition of enzymatic and binding activities, increased susceptibility to aggregation and proteolysis, modifications in uptake by cells, and altered immunogenicity (Shafer, 2000).

2.2.3. Lipids

In the presence of ROS/RNS and oxygen, lipids undergo a chain oxidation reaction leading to peroxidation. This chain reaction is initiated by oxidants, primarily the hydroxyl radical and leads to the formation
of cellular responses. Additionally, these oxidants also in oxidization of proteins, lipids and DNA (Taraseviciene-Stewart & Voelkel, 2012), can result in direct lung damage or induce an assortment of oxidative stress.

2.3. Effects of oxidative stress

ROS and RNS normally play roles in cell signalling and homeostasis; however, in excess they become increasingly toxic. The ROS and RNS (e.g., vitamin E) (Kalyanaraman, 2013) (Fig. 4). This is especially important in comorbid cardiovascular disease where the formation of plaques in the arteries is initiated from lipid peroxidation.

2.3.1. Systemic oxidative stress

Even after smoke cessation, systemic oxidative stress still persists in COPD patients. This is reflected by an elevation in circulating ROS and a depletion of antioxidants. The increased oxidative stress is likely due to the persistent low grade systemic inflammation resulting in the production of ROS systemically (Foschino Barbaro et al., 2007; Dalal, Shah, Lunacek, & Hanania, 2011; Zampetaki, Dudek, & Mayr, 2013). In addition to the links between oxidative stress in COPD and comorbid diseases, increased extrapulmonary oxidative stress is also an independent risk factor of many comorbidities such as cardiovascular disease, osteoporosis and depressive disorders in patients without COPD (Sanchez-Rodriguez, Ruiz-Ramos, Correa-Munoz, & Mendoza-Nunez, 2007; Kawada, 2012; Michel, Pulschen, & Thome, 2012).

2.4. Oxidative stress in comorbidities of chronic obstructive pulmonary disease

A prominent feature of COPD is the presence of comorbid diseases (or comorbidities) in patients (Chatila et al., 2008; Patel & Hurst, 2011). These diseases can range from more “mild” conditions that affect quality of life such as osteoporosis and depression, to more fatal diseases such as ischaemic heart disease and cancer. COPD-related mortality is likely underestimated due to these comorbidities as their presence makes it difficult to determine the precise cause of death. Respiratory failure is considered to be the major cause of death in advanced COPD; however, comorbidities such as cardiovascular disease and lung cancer are also major causes of death. In early/mid stages of COPD, comorbidities are the leading causes of mortality (Sin et al., 2006). Comprehensive studies on the specific cause of deaths in COPD patients have found that rather than dying from progressive respiratory failure, many COPD patients die from a complex web of interconnected comorbidities (van Eeden & Sin, 2013). For the purpose of this review, comorbidities are defined as the presence of one or more distinct diseases or disorders that are not directly related to COPD or are not part of the natural history/progression of the disease (e.g., respiratory infections resulting in AECDP). The underlying mechanisms resulting in these systemic manifestations in COPD are not fully understood; however, they have been linked to the persistent low-grade systemic inflammation and persistent oxidative stress in COPD as discussed earlier.

3. Cardiovascular disease (CVD)

3.1. Cardiovascular disease overview

CVD is a broad term used to describe any disease involving the heart and/or blood vessels. The presence of CVD is very common in COPD patients and is the leading cause of morbidity and mortality in younger patients and patients with mild-to-moderate COPD (Sin et al., 2006). Comorbid CVD can manifest itself in one or more various disorders such as angina, stroke, arrhythmias, hypertrophy of the heart, and myocardial infarction, and its presence greatly reduces the survivability of COPD patients (Dalal et al., 2011).
3.1.1. Cardiovascular disease and chronic obstructive pulmonary disease links

It is well documented that COPD and CVD share many of the same risk factors, such as smoking, diet, and pre-existing hypertension. When considering these risk factors it is of significance that many, if not all, contribute to disease progression at least in part via oxidative stress. Multiple studies have shown links between COPD and classical cardiovascular risk factors such as family history of coronary heart disease and diabetes (Sidney et al., 2005; Bursi, Vassallo, Weston, Killian, & Roger, 2010; Terzano et al., 2010; Ford et al., 2012). Persistent low-grade systemic inflammation is present in both COPD and CVD and oxidative stress, which plays a major role in COPD, has also been implicated in CVD (Zampetaki et al., 2013). This indicates that the systemic inflammation and increased oxidative stress in COPD may lead to the onset and development of comorbid CVD.

In addition to this, acute inflammatory lung conditions, such as bacterial/viral infection or acute exposure to airborne irritants, are also associated with vascular dysfunction (Mills et al., 2005, 2007). Increased systemic inflammation, as a result of these lung conditions, is capable of destabilizing vulnerable plaques, inducing a prothrombotic state (Man, Van Eeden & Sin, 2012). COPD patients generally do not tolerate cardiac injury or intervention as well as healthy individuals. COPD patients with acute myocardial infarction have a five-year survival rate of 46% compared to 68% in those without COPD (Bursi et al., 2010).

3.1.2. Cardiovascular disease and acute exacerbations of chronic obstructive pulmonary disease

Although many factors have been associated with poor outcomes from AECOPD, CVD is becoming increasingly recognized as a strong predictor of in-hospital mortality. Studies have shown that over 50% of patients hospitalized for AECOPD have a high prevalence of coexisting cardiovascular disease (Stefanelli et al., 2013). Comorbid cardiovascular disease is independently associated with increased risk of AECOPD (Masunaga et al., 2003). The severity of airway obstruction is a major predictor of AECOPD, and is also an independent risk factor for cardiovascular disease (Roberts et al., 2002; Mannino & Davis, 2006; Steer et al., 2010; Mannino, 2011). There is also mounting evidence associating a high frequency of acute CVD with acute respiratory illness, such as pneumonia or AECOPD. Studies have shown that in the general population, subjects with respiratory tract infections are more likely to experience a myocardial infarction within 2 weeks of infection (Meier, Jick, Derby, Vasilakis, & Jick, 1998; Corrales-Medina, Madjid, & Musher, 2010). A retrospective review examining 24 h mortality following AECOPD hospitalisation found that approximately 60% of deaths that occurred resulted from cardiovascular causes (Pastor et al., 2013).

3.1.3. Pulmonary hypertension, hypertrophy and heart failure

There are two principal pathological features in the pulmonary vascular architecture common to most forms of pulmonary hypertension: excessive vasoconstriction and remodelling of the pulmonary arteriolar wall, which primarily occur by a mechanism of smooth muscle proliferation within the medial layer (Demarco, Whaley-Connell, Sowers, Habibi, & Dellsperger, 2010). Because ROS may promote vasoconstriction, smooth muscle cell proliferation, and vascular remodelling, oxidative stress likely plays a critical role in many forms of pulmonary hypertension.

Multiple studies have shown that 25–70% of COPD patients have pulmonary hypertension, depending on the definition used (Rasubala, Yoshikawa, Nagata, Iijima, & Ohishi, 2003; Sekine et al., 2014). In addition to this, it is estimated that 25% of patients with moderate-to-severe COPD develop pulmonary hypertension within 6 years if they have no hypertension at baseline (Zhai, Yu, Wei, Su, & Christiani, 2014). The pathological changes implicated in the development of pulmonary hypertension can also be seen in tissue samples of COPD patients who do not have a diagnosis of pulmonary hypertension (Weitzenblum, 1984; Santos et al., 2002).

A major feature of COPD is emphysema, contributing to airway obstruction. The destruction of alveolar walls and enlargement of airspaces results in a reduction in gas exchange (van der Toorn et al., 2007). To compensate for this, blood pressure is increased to facilitate more pulmonary blood flow leading to pulmonary hypertension. This results in increased work in the right heart and over time this increased workload may lead to concentric hypertrophy of the right ventricle (RV) (Neofytou, Tzortzaki, Chatziantoniou, & Siafakas, 2012). This can lead to reduced ejection fraction, elevated end diastolic RV pressure, impediment of the right heart and, if left unchecked, can lead to heart failure. These changes can be detected in COPD patients, especially in end-stage COPD patients, using transthoracic echocardiography, which allows for the measurement of various parameters in the heart such as systolic and diastolic wall thickness, and chamber size. Both pulmonary hypertension and right heart failure are associated with an increase in morbidity and mortality in COPD patients, independent of the decline in lung function (Stone, Machan, Mazer, Casserly, & Klinger, 2011).

3.2. Oxidative stress in cardiovascular disease

The presence of oxidative stress in the form of increased ROS/RNS formation (e.g., O₂−, ONOO−) has been observed both clinically and in animal models of CVD (Schnabel & Blankenberg, 2007; Afanas’ev, 2011). Although implicated in CVD, the cause–effect relationship of oxidative stress with any of the different cardiovascular diseases has yet to be established. The increased generation of ROS due to impaired mitochondrial reduction of molecular oxygen, secretion of ROS by inflammatory cells, endothelial dysfunction, auto-oxidation of catecholamines, as well as exposure to radiation or air pollution can drive the oxidative stress in cardiac and vascular myocytes (Dhalla, Temshah, & Netticadan, 2000). In addition to the increased oxidant production, depression in the antioxidant reserve has also been implicated in CVD. These typically phenolic antioxidants (e.g., vitamin E) act as a protective mechanism in cardiac and vascular myocytes, and their reduced levels appear to be due to both the saturation by excess ROS/RNS and/or changes in gene expression. The harmful effects caused by ROS/RNS in cardiovascular tissues are mainly due to ability of the oxidant species to drive changes in subcellular organelles (e.g., mitochondria), reduce NO+ bioavailability, and induce intracellular Ca²⁺ overload (Dhalla et al., 2000).

3.2.1. Cardiovascular generation of reactive oxygen species/reactive nitrogen species

As stated earlier, in addition to phagocytising cells, NOX-dependent ROS-generation has been observed in numerous non-phagocytising cells, though at a lower level. In the cardiovascular system these include vascular smooth muscle cells (VSMCs), endothelial cells, adventitial and cardiac fibroblasts and cardiomyocytes (Cave, Grieve, Johar, Zhang, & Shah, 2005). Normally, these cells continuously generate low levels of ROS even in the absence of external stimuli, and ROS derived from vascular NOX act as second messengers in VSMC signalling. Over long periods of time, vascular NOX complexes only produce low levels of O₂−; with much of it generated intracellularly where it participates in cell signalling (compared to phagocyte O₂− which is generated extracellularly) (Fisher, 2009). When stimulated however, there is a significant increase in NOX-driven ROS production by these cells. It is important to recognize that both vascular and phagocytic NOX play an important role in superoxide production as phagocytes can infiltrate cardiovascular tissues and facilitate the functional and structural alterations observed in CVD (Matoba & Egashira, 2011).

3.2.2. Oxidative stress in the pathogenesis of cardiovascular disease

Damage to the endothelium is the initiating step in CVD. This damage can expose endothelial cells, along with the underlying cell layers, to the deleterious effects of the inflammatory process, which can ultimately lead to the formation of atherosclerotic lesions. Cellular oxidative stress caused by excess ROS/RNS production is considered to be
intrinsic to atherosclerotic lesion formation (Vogiatzi, Tououlis, & Stefanadis, 2009). Exogenous factors contributing to oxidative stress such as smoking and comorbid diabetes also contribute to vascular oxidative stress and are strong risk factors for CVD (Fearon & Faux, 2009). Cigarette smoke has been associated with the down-regulation of key exogenous and endogenous antioxidants such as vitamin C (ascorbic acid), carotene, Gpx and SOD (Tsuchiya et al., 2002; Agnihotri et al., 2009). This can lead to dysfunction in endothelial cells, monocytes and VSMCs as well as mitochondrial damage. In addition to this, DNA damage can be caused by oxidised lipids and this may also contribute to the dysfunction of endothelial cells, VSMCs, T lymphocytes and macrophages (Madamanchi & Runge, 2007).

Oxidative stress has also been associated with the apoptosis or programmed cell death of cardiac myocytes (Singal, Khaper, Palace, & Kumar, 1998). The loss of myocytes via apoptosis has been observed in the infarct regions of myocardium from patients that have suffered from heart failure or a myocardial infarction (MI) (Krijnen et al., 2002). Both in vitro studies and in vivo animal model studies found that apoptosis occurs in response to cardiovascular complications, such as MI, and chronic pressure overload (Fiorillo et al., 2005). The common factor in all of these conditions is the generation of oxidative stress and oxidative stress is known to play a role in the initiation of apoptosis (Nagata et al., 2003). Additionally, the apoptosis of myocytes is inhibited by antioxidants such as vitamin E and SOD, implicating ROS/RNS in the pathological pathways of CVD (Kumar, Lou, & Singal, 2002). Although studies have implicated a role for ROS/RNS in CVD related apoptosis, the exact contribution of oxidative stress in the loss of myocardial function and heart failure remains to be established.

3.2.3. Nitric oxide in cardiovascular disease

The role of nitric oxide NO in vascular homeostasis and signalling has been well characterized. As stated earlier, NO plays a pivotal role in the maintenance of vascular tone and vasoreactivity. In contrast to this distinct role in cell physiology, NO can also contribute to CVD pathology. Under certain conditions eNOS (the endothelial isoform of the nitric oxide synthase) becomes uncoupled from a NO− to ft h e s e treatments and antioxidant therapies

The current treatments for comorbid CVD are the same treatments used for CVD independent of COPD, such as β receptor blockers, ACE inhibitors, or angiotensin receptor blockers. However, the benefit of these drugs in COPD patients with CVD is conflicting. Some studies have found that these treatments may be detrimental for COPD patients, worsening the pulmonary symptoms of the disease (Nojiri et al., 2014). However, others have shown that when given to COPD patients with and without comorbid COPD, these treatments have shown notable benefits with regards to airway symptoms (Huang et al., 2013). This may be due to the fact that both ACE inhibitors and angiotensin receptor blockers have displayed pleiotropic antioxidant effects in addition to their anti-hypertensive effects (Munger, 2011). Thus, this presents a unique opportunity for dual action therapy using CVD-specific treatments and antioxidants, and obviously warrants further research.

CVD and COPD also overlap with regards to their non-pharmacological management. Cardiovascular modifying factors such as physical activity and diet modification are reported to decrease hospital readmission for COPD (McLachlan, Hambl, Almsherqi, El Oakley, & McGuire, 2006). The recognition of the importance of oxidative stress in CVD has led to the fervent use of antioxidants in the treatment and prevention of the disease; however, the results of prospective, randomized clinical trials have been generally disappointing (Myung et al., 2013; Ye, Li, & Yuan, 2013). In contradiction, studies have shown that antioxidant therapy is beneficial in non-comorbid hypertension, atherosclerosis, ischaemic heart disease, cardiomyopathies and congestive heart failure (Dhalla et al., 2000). It should be noted that almost all antioxidant clinical studies only explored the effectiveness of traditional scavenging antioxidants such as vitamin E.

With regards to antioxidant treatments, the protective role of exercise in preventing oxidative stress is noteworthy. Acutely, it is known that exercise causes oxidative stress (Fisher-Wellman & Bloomer, 2009). However, exercise also leads to longer-term activation and enhanced synthesis of antioxidants and antioxidant enzymes (e.g., SOD, Gpx-1), as well as decreasing oxidant production (Gonzalez, Marquina, Rondon, Rodriguez-Malaver, & Reyes, 2008). This may explain the links between prolonged exercise and the beneficial effects to both COPD and CVD patients, and highlights the potential benefits of targeting oxidative stress in comorbid CVD.

Overall, it appears that targeting oxidative stress with antioxidant enzyme modifying treatments may have great potential in COPD and comorbid CVD by providing beneficial effects with regards to both the pulmonary and cardiovascular aspects. 4. Skeletal muscle wasting

4.1. Skeletal muscle wasting overview

Skeletal muscle wasting, also referred to as cachexia or skeletal muscle atrophy, occurs in approximately 20 to 40% of all COPD patients (Schols et al., 1998; Congleton, 1999; Maltais et al., 2014). Muscle wasting is characterized by a marked decrease in skeletal muscle mass, an increase in proportion of type 1 muscle fibres in the diaphragm and type 2 muscle fibres in the periphery, and associated with low exercise capacity and skeletal muscle weakness. Skeletal muscle wasting in COPD is a strong predictor of mortality, independent of decline in lung function (Schols et al., 1998; Marquis et al., 2002; Swallow et al., 2007). Although present in a large population of patients, the prevalence of muscle wasting can only be approximated as there are no simple techniques to measure muscle mass. Knowing this, the actual prevalence and extent of muscle wasting in the COPD population are likely underestimated. This is because all the data is extrapolated from body weight measurements and lean body mass, an index of muscle mass, may be reduced despite the preservation of total body weight. This is further supported by the fact that patients have a proportionally greater reduction in thigh muscle cross-sectional area compared to reduction in body weight (Marquis et al., 2002). This indicates that a preferential loss of muscle tissue exists in emaciated patients with COPD (Kim et al., 2008).

Skeletal muscle wasting, although not a directly fatal condition, reduces health-related quality of life and decreases survivability for COPD patients. The main feature of comorbid skeletal muscle wasting is a reduction in fat free mass (FFM) and is associated with weaker peripheral muscles, impaired functional status, as well as poor health-related quality of life (Debiegare, Cote, & Maltais, 2001; Mathur, Brooks, & Carvalho, 2014). In COPD, the reduction in muscle mass is proportional to that of the reduction in strength indicating that the remaining contractile apparatus may be functionally preserved. However, in patients chronically treated with glucocorticosteroids, it is possible for the loss of strength to be disproportional to the reduction in muscle mass.

Deteriorations in FFM have also been described following acute exacerbations. These COPD patients also experience a reduction in exercise capabilities, with skeletal muscle alterations contributing to limitations in exercise in addition to pulmonary dysfunction (Wust & Degens, 2007). It should be noted that the strength of the quadriceps is a key determinant of exercise tolerance in COPD. This is explained by the influence that muscle strength has on the perceived leg effort
required during exercise, which is considered to be the main limiting symptom in 40–45% of patients with COPD (Debigare & Maltais, 2008).

4.1.1. Mechanisms of skeletal muscle dysfunction

The pathophysiological interaction between COPD and alterations in skeletal muscle tissue is poorly understood and represents an important gap in knowledge of the disease. Skeletal muscle wasting in COPD is multifactorial in nature with several of these factors likely interacting. Many factors (e.g., inflammation, oxidative stress, and poor nutrition) can initiate or enhance alterations in skeletal muscle, such as change in fibre type phenotypic expression and regenerative defects in peripheral muscles of patients with COPD (Maltais et al., 2014). In this review, multiple risk factors will be briefly discussed along with a more in-depth discussion regarding the role of oxidative stress and ROS.

4.1.1.1. Smoking. Cigarette smoking or exposure to other airborne irritants is unlikely to be the primary mechanism involved in skeletal muscle wasting in COPD, as seen in several studies where patients and control subjects were matched for smoking history (Maltais et al., 2014). Nevertheless, smoking does have some effect on muscle biology and it may predispose patients to the development of skeletal muscle dysfunction (De Paepe et al., 2008; Rinaldi et al., 2012). Smoking is also associated with skeletal muscle weakness in otherwise healthy individuals (Seymour et al., 2010; Barreiro et al., 2011; van den Borst et al., 2011).

4.1.1.2. Disuse. Peripheral (or limb) muscle dysfunction in COPD has been partly attributed to a reduction in physical activity, or “deconditioning.” In general, the disuse of muscle can lead to many of the features and alterations of skeletal muscle in COPD patients: muscle weakness, muscle atrophy, loss of type I fibres, decreased cross-sectional area of muscle fibres, reduced oxidative enzyme activity, reduced capillary-to-fibre ratio, early lactate release, reduced rate of phosphocreatine synthesis after exercise and altered redox status (Booth & Gollnick, 1983; Coyle, Martin, Bloomfield, Lowry, & Holloszy, 1985; Larsson & Ansved, 1985; Polkey & Moxham, 2006). In healthy adults, these changes are fully reversible in response to training and increased exercise; however, full recovery is unusual in COPD patients (Troosters, Gosselink, & Decramer, 2000; Polkey & Moxham, 2006, 2011; Man, Kemp, Moxham & Polkey, 2009).

4.1.1.3. Inflammation. A low body mass index (BMI) has been linked to systemic inflammation in COPD patients. As seen in Fig. 5, inflammation plays a key role in the activation of protein breakdown in skeletal muscle. Inflammation results in the production of key cytokines, such as IL-8, that can generate an array of cellular responses. This includes the induction of the ubiquitin proteasome (UbP) system through the transcriptional activities of NF-κB and FOXOs, apoptosis, and macroautophagy, all of which have been linked to the muscle atrophy (Kandarian & Jackman, 2006). In COPD patients, there is a lack of evidence with regards to inflammation in skeletal muscle during stable disease (Gosker et al., 2003; Montes de Oca et al., 2005); however, increased inflammation is seen during periods of exacerbation (Spruit et al., 2003; Yende et al., 2006). Because of this, the role of inflammation as the key event for the development of skeletal muscle dysfunction in COPD is still widely debated.

4.1.1.4. Hypoxia. In humans (Hoppeler et al., 1990) and animals (Magalhaes et al., 2005) muscle mass decreases under hypoxic conditions. COPD patients that have low arterial O$_2$ and reduced O$_2$ delivery tend to have lower body mass than those with normal levels of arterial O$_2$ and sufficient O$_2$ delivery (Semenza, 2009). Hypoxia may be a factor driving changes in limb muscle tissue as hypoxia can induce downstream effects leading to the activation of the UbP system and reduced myogenesis (Caron, Theriault, Pare, Maltais, & Debigare, 2009).

4.2. Oxidative stress in skeletal muscle wasting

In addition to chronic inflammation, hypoxia, cigarette smoke, sepsis and an increased cost of breathing cause the increased generation of oxidants in the lungs (e.g., H$_2$O$_2$, O$_2^•$ − , MDA) (Rahman, 2005). It is suggested that, in addition to inflammatory mediators, these oxidants can

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**Fig. 5.** Regulation of muscle mass relies on various hypertrophy and atrophy signalling pathways. In skeletal muscle wasting, there is an imbalance between these anabolic and catabolic processes resulting in enhanced muscle degradation as a result of protein breakdown. Myostatin (a negative regulator of muscle mass) can inhibit muscle growth. Inflammatory mediators and ROS can lead to the downstream activation of atrophy-related genes (e.g., Atrogin-1, MuRF-1) resulting in enhanced protein degradation. Inflammation and oxidative stress have also been implicated in the activation of the ubiquitin–proteasome (UbP) system. The UbP system is the primary mechanism of the protein catabolism in mammalian skeletal muscle. Refer to Maltais et al. (2014) for an exhaustive overview of the signalling pathways involved.
spill into circulation, increasing the systemic oxidative stress burden of COPD patients (Zeng et al., 2013). In vivo models of skeletal muscle wasting in COPD, increased oxidative stress has been observed (Rinaldi et al., 2012). This increase in oxidative stress can modify muscle proteins, reducing their integrity and enhancing their degradation (Aiken, Kaake, Wang, & Huang, 2011). Direct exposure to oxidative stress from environmentally-derived oxidants, or indirect exposure via cellular-derived ROS due to inflammation, can induce proteolysis. Acute bouts of physical exercise and acute exacerbations can also increase the level of oxidative stress (Couillard et al., 2003; Karadag, Karul, Cildag, Yilmaz, & Ozcan, 2008; Stanojkovic et al., 2011). It is suggested that oxidative stress may acutely affect skeletal muscle function by inhibiting the activity of the sodium/potassium pump, sarcoplasmic reticulum function, myosin ATPase and mitochondrial respiration (Wust & Degens, 2007). In addition to these acute effects, chronic oxidative stress also contributes to muscle wasting and dysfunction in both respiratory and peripheral muscles. The increased presence of ROS associated with COPD patients with muscle wasting experience a more severe abnormal oxidative stress response to submaximal and maximal exercises compared to non-muscle-wasted patients with COPD (Van Helvoort et al., 2006). Although most studies focus on the increased signalling of atrophy pathways as the major driving force of comorbid skeletal muscle wasting, it should be noted that decreases in hypertrophy signalling may also contribute greatly to the pathology of the disease. Further studies are required to determine the exact contribution of these various pathways to the disease.

4.3. Current treatments and antioxidant therapies

There are currently no effective drug treatments for skeletal muscle wasting in COPD. Pharmacological treatments that have been explored include anabolic steroids, growth hormone, other growth anabolic compounds and bioactive nutrients (e.g., ghrelin, creatine) and antioxidants. Most of these treatments have only shown little to modest benefits when treating skeletal muscle wasting and weakness in COPD. As with CVD, skeletal muscle wasting and COPD also overlap with regards to their non-pharmacological management. Currently, the most potent treatment for limb muscle dysfunction is exercise training, which is already a key component for the management of COPD (Maltais et al., 2014). Some treatments such as lifestyle modifications (diet and exercise) and anabolic steroids have shown improvement in patients; however, they do not reverse the progression of muscle loss and may only increase muscle growth without substantial improvements in strength or endurance (Casaburi et al., 2004; Hansen, Gualano, Bozinovski, Vlahos, & Anderson, 2006). Therefore, new treatments are needed as limiting muscle wasting in COPD will lead to improved quality of life and increased survivability of patients. Because of its role in the pathogenesis of comorbid skeletal muscle wasting, targeting oxidative stress may be able to slow the progression or onset of the disease, giving patients an improved quality of life whilst managing their other symptoms. Furthermore, it has been reported that increasing antioxidant potential can improve muscle performance whilst attenuating muscle fatigue (Maltais et al., 2014). Further pre-clinical and clinical studies are required to determine the effectiveness of targeting oxidative stress in comorbid skeletal muscle wasting.

5. Lung cancer

5.1. Lung cancer overview

Lung cancer is one of the more fatal comorbidities of COPD. It is currently the most frequently diagnosed cancer worldwide and is the number one cause of cancer-related deaths in males (Jemal et al., 2011). Lung cancer accounts for up to 13% of all cancer diagnoses worldwide, accounting for more than one million deaths per year (Alberg &
Nonemaker, 2008; WHO, 2013). Patients with COPD have a higher risk of developing lung cancer and due to the presence of comorbid lung cancer, the survival rate of these COPD patients is drastically low (Raviv, Hawkins, DeCamp, & Kalhan, 2011). It is estimated that up to 70% of lung cancer patients have co-existing COPD, and one study found that the most common cause of death among patients with airflow obstruction was lung cancer (Lung Health Study Research, 2000). It is also known that, whilst lung cancer survival in the general population is very low, COPD patients with comorbid lung cancer have an even lower rate of survival. In one study, lung cancer patients without COPD had a 26% 3 year survival after diagnosis versus 15% in lung cancer patients with COPD (Kiri, Soriano, Visick, & Fabbri, 2010).

1.5.1. Lung cancer and chronic obstructive pulmonary disease links

It is well established that exposure to airborne irritants, especially those in cigarette smoke, induces both diseases. Cigarette smoke, among other airborne irritants, is an independent risk factor of both COPD and lung cancer with approximately 80% and 90% of cases associated with cigarette smoking respectively (Fathy, Hamed, Youssif, Fawzy, & Ashour, 2014). Additionally, almost 1% of COPD patients develop lung cancer yearly, which may be associated with genetic susceptibility to particulates found in cigarette smoke (Sekine, Katsura, Koh, Hiroshima, & Fujisawa, 2012). COPD is also considered to be a risk factor for lung cancer independent of smoking status. It has also been suggested that even a small reduction in airflow significantly predicted lung cancer (Wilson et al., 2008). The Multiple Risk Factor Intervention Trial reported that a 10% reduction in lung function was associated with an almost 3-times greater risk of lung cancer compared to patients without airflow limitations, after adjusting for smoking (Shaten et al., 1997). It also indicated that the lag time between smoking cessation and the beneficial effects on lung cancer development may be as long as 20 years. A separate study found that the presence of COPD increases the risk of lung cancer by up to 4.5-fold (Wang, 2013). This indicates that COPD patients have a greater risk of developing lung cancer compared to those with normal pulmonary function regardless of smoker status. On the other hand, a study looking at the prevalence of COPD in lung cancer patients, independent of age, sex, or smoking history, determined that the prevalence of pre-existing COPD in recently diagnosed lung cancer cases was six-times greater than in smokers without lung cancer (Young et al., 2009). The study found that approximately 50% of newly diagnosed lung cancer patients had COPD, whereas this number was only 8% in otherwise healthy smokers. This suggests that impaired lung function may be more important than age or smoking history as a predictor of lung cancer.

Whilst the association between lung cancer and COPD has always been of great interest, the details of their various molecular pathways involved and their clinical correlates have only begun to be established. Chronic inflammation is known to induce both COPD and lung cancer by means of the release of inflammatory mediators and the excess production of ROS/RNS (Lawless, O’Byrne, & Gray, 2009; Lee, Walser & Dubinett, 2009; Bozinovski et al., 2015). Inflammatory mediators may promote the growth of bronchoalveolar stem cells, activation of NF-κB and signal transducer and activation of transcription 3 (STAT3), which all play crucial roles in the development of lung cancer (Sekine et al., 2012). Chronic inflammation leads to increased ROS production, and increased oxidative stress has also been associated with the development of lung cancer (Barreiro et al., 2013).

5.2. Oxidative stress in lung cancer

The primary association between oxidative stress and the development of lung cancer is the oxidative modifications to DNA as a result of ROS/RNS (Cooke et al., 2003; Paz-Elizur et al., 2003). Oxidative damage to DNA by ROS and RNS has been implicated in carcinogenesis (Cooke et al., 2003). As stated earlier it is known that oxidant-induced DNA damage also involves single- or double-stranded DNA breaks and DNA cross-links, DNA damage inducing either arrest or induction of transcription, induction of signal transduction pathways, replication errors, and genomic instability, all of which have been linked to carcinogenesis (Dawane & Pandit, 2012). It should be noted that studies have found that excessive amounts of NO+ increase apoptosis in some tumour cells, whereas reduced amounts of NO+ can increase the vascularity of the tumour and protect the cells from apoptosis, particularly in lung cancer (Masri, 2010).

Due to the direct damage to DNA and the increased demand for DNA repair as a result of oxidative stress, more DNA mutations may occur. One of the most important oxidative modifications to DNA is the adduct of •OH on DNA nucleobases. The 8-hydroxy-2′-deoxyguanosine (8-OHdG) and/or its tautomeric 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) have been widely studied and proven important mutagenic adducts to DNA and are therefore key biomarkers of oxidative stress induced carcinogenesis (Valavanidis et al., 2009a,2009b). Mutations of 8-oxodG involve a GC→AT transversion which can modify protein transcription as stated earlier in the review. In addition to this, ROS and RNS may initiate carcinogenesis by attacking DNA nucleobases causing changes in oncogenes and tumour suppressor genes (Valavanidis, Vlachogiannii, Fiotakis, & Loridas, 2013). Studies found that •OH can generate various DNA phenotypes with various metastatic potentials (Malins, Polissar, & Gusselman, 1996; Valavanidis et al., 2013). It is suggested that these different phenotypes likely contribute to the diverse physiological properties and heterogeneity characteristic of metastatic cell population (Malins et al., 1996).

In addition to direct damage to DNA, ROS and RNS can indirectly damage DNA via oxidative modifications to lipids. This occurs when ROS and/or RNS react with cellular membrane phospholipids generating lipoperoxide radicals (LOO•) and toxic aldehydes (malondialdehyde, MDA). This results in membrane permeability and microcirculation alteration and also causes the activation of nuclear factors leading to other pro-inflammatory agents (Valavanidis et al., 2013). MDA is a naturally occurring by-product of lipid peroxidation and is a known mutagenic and carcinogenic compound. MDA reacts with DNA to form multiple adducts with mutagenic potential (Marnett, 2000). Additionally, MDA itself has also been shown to be carcinogenic (Marnett & Tuttle, 1980).

As with patients with COPD and comorbid lung cancer, patients without co-existing COPD also experience an increase in systemic oxidative stress and inflammation (Barreiro et al., 2013). These processes can cause the activation of NF-κB, which has been associated with the development of comorbid lung cancer (Chen, Li, Bai, & Lin, 2011). NF-κB activation and the subsequent actions of inflammatory-related genes may play a central role in both COPD and lung cancer. Activation of NF-κB increases the release of inflammatory mediators that can induce COPD, whilst also inhibiting apoptosis, inducing proliferation and, accelerating cancer development (Karim, 2009). The release of these inflammatory mediators can activate STAT3 which is a transcription factor with various physiological functions. Continuous activation of the STAT3 signaling pathway can potentiate pulmonary inflammation and induce adenocarcinoma formation in the lung (Takata et al., 2012). Clinically, activation of STAT3 and its downstream genes serve as biomarkers of COPD and lung cancer diagnosis and prognosis (Qu et al., 2009).

As stated previously, oxidative stress can stimulate the recruitment of macrophages and neutrophils which release various cytokines and chemokines. Released matrix metalloproteinases and RNS promote inflammation, induce apoptosis, matrix degradation, and ineffective tissue repair, resulting in enlarged airspaces (empysema) (Belvisi & Bottomley, 2003). Bronchoalveolar stem cells may attempt to repair and replace damaged alveolar cells however, in an inflammatory environment, they can enhance carcinogenesis (Sekine et al., 2012).

5.3. Current treatments and antioxidant therapies

Treatment for lung cancer, regardless of co-existing COPD, depends on the type and severity of the cancer with the main treatments being surgery, chemotherapy and radiotherapy in varying combinations
Osteoporosis is a progressive, metabolic bone disease that is characterized by a decrease in bone mass and density which leads to a deterioration in bone strength and an increased risk of fracture (Kanis et al., 2013). Patients with osteoporosis experience reduced bone mineral density (BMD) and deterioration of bone microarchitecture (Kanis, 2002). Additionally, patients experience alterations to the amount and variety of proteins in bone as a result of the disease (Donoso, Pino, Seitz, Osses, & Rodriguez, 2015). Osteoporosis presents no actual symptoms; however, the major consequence of osteoporosis is the increased risk of bone fractures as a result of the bone alterations. The key difference between normal bone fractures and osteoporotic fractures is that osteoporotic fractures occur in scenarios where healthy individuals would not normally break a bone. Therefore, these fractures are often regarded as fragility fractures. Generally, these fragility fractures occur in the vertebral column, ribs, hips, and wrists (Adachi et al., 2003; Leslie et al., 2014).

As bone is a dynamic tissue, it continuously renews itself throughout life and this is accomplished via bone remodelling. This process is carried out by a functional and anatomic structure known as the basic multicellular unit. This structure requires the coordinated action of three major types of bone cells: osteoclasts, osteoblasts and osteocytes (Feng & McDonald, 2011). The interactions between these cells and multiple molecular mediators, including hormones, growth factors, and cytokines, drive the remodelling process (Mundy, 1993). Bone remodelling follows a time sequence that lasts approximately six months in humans and during this process, osteoclasts eliminate old and/or damaged bone which is then replaced by new bone formed by osteoblasts. Osteocytes are relatively inert cells, however, they are primarily responsible for the molecular synthesis and modification, and transduction of signals (akin to the nervous system) necessary to sustain the activity of alkaline phosphatase (ALP), have been identified in muscle wasting leading to significant reductions in FFM. The increased enzyme catalytic activity in muscle wasting is also associated with bone loss. Significant changes in enzyme function, such as increased activity of alkaline phosphatase (ALP), have been identified in sera collected from osteoporosis patients (Leung, Fung, Sher, Li, & Lee, 1993). These changes have also been associated with increased bone metabolism.
and altered bone rearrangement in COPD (Jorgensen & Schwarz, 2008). MMP activity (primarily MMP-9 and MMP-12), which is implicated in parenchyma destruction, has also been linked to the development of osteoporosis in COPD patients (Bolton et al., 2009).

6.2. Oxidative stress in osteoporosis

In addition to inflammation, there have been a multitude of studies implicating oxidative stress with an increased rate of bone loss, thus making oxidative stress a risk factor for osteoporosis (Varanasi, Francis, Berger, Papiha, & Datta, 1999; Chavan, More, Mulgund, Saxena, & Sontakke, 2007; Stanokjovic et al., 2013; Cervellati et al., 2014). It is suggested that ageing and the consequential increase in ROS are responsible for bone loss (Almeida & O’Brien, 2013). Increased oxidative stress is associated with alterations in the activity and function of both osteoblast and osteoclast cells; the two major bone cells involved in the pathogenesis of osteoporosis.

6.2.1. Osteoblasts

The role of oxidative stress in osteoblasts has only been researched extensively in the past decade. As stated earlier, osteoblasts are responsible for replacing old or damaged bone with new bone. Studies found that osteoblasts can be induced to produce intracellular ROS (\(O_2^-\) and \(H_2O_2\)) and the production of which can cause a decrease in ALP which can cause cell death, and this effect is partially inhibited by scavenging antioxidants (Darden, Ries, Wolf, Rodriguez, & Key, 1996; Onyia et al., 1999). In high concentrations, ROS can damage osteoblast cells, preventing the normal growth and development of bone, and has been shown to induce osteoblast cell death (Lee, Lim, Lee & Yang, 2006). Additionally, \(H_2O_2\) can modulate the activity of intracellular calcium in osteoblasts by increasing the amount of \(Ca^{2+}\) released from intracellular \(Ca^{2+}\) stores (Nam, Jung, Yoo, Ahn, & Suh, 2002).

6.2.2. Osteoclasts

Whilst still not well understood, the different mechanisms and pathways involved in the differentiation of osteoclasts and their ability to resorb bone are beginning to be elucidated. It is well known that ROS is involved in this process, but the extent of its contribution is still to be determined. Superoxide has been detected intracellularly in osteoclasts and at the osteoclast–bone interface, suggesting a contribution from superoxide in bone resorption (Key, Wolf, Gundberg, & Ries, 1994). It is known that osteoclastic superoxide is produced by NOX (Darden et al., 1996). \(H_2O_2\) produced by endothelial cells associated with osteoclasts and the \(H_2O_2\) produced by osteoblasts have been shown to increase osteoclastic activity and bone resorption, which may contribute to the increased breakdown of bone in osteoporosis (Bax et al., 1992). \(H_2O_2\) has also been implicated in various other osteoclast functions such as osteoclast motility, differentiation of osteoclast precursors and the regulation of osteoclast formation (Steinbeck, Kim, Trudell, Hauenschlak, & Kurnovsky, 1998). The reaction of \(H_2O_2\) with the tartrate-resistant acid phosphatase found on the surface of osteoclasts is responsible for the degradation of collagen and other proteins (Bull, Murray, Thomas, Fraser, & Nelson, 2002). Additionally, antioxidants play a role in osteoclast activity. Osteoclasts innately possess the antioxidant enzyme SOD in the plasma membrane (Steinbeck, Appel, Verhoeven, & Kurnovsky, 1994). Studies found that after treating osteoclast cells with antioxidant enzymes such as SOD and catalase, ROS production is inhibited indicating that antioxidant therapy may be beneficial for patients with osteoporosis (Lee, Kim & Jing, 2014).

6.3. Current treatments and antioxidant therapies

In addition to diet supplementation with calcium and vitamin D, there is also a wide range of pharmaceuticals available for the treatment of osteoporosis. The current anti-resorptive treatments prescribed to osteoporosis patients include a number of bisphosphonates which inhibit bone resorption (MacLean et al., 2008). These vary by way of administration and are taken orally either daily, weekly, monthly or intermittently. Many other drugs are available such as, but not limited to, calcitriol and strontium ranelate, both of which increase bone formation by stimulating osteoblasts, whilst reducing bone resorption via inhibiting the activity of osteoclasts. Emerging research has shown beneficial effects of anabolic treatments in osteoporosis (Neer et al., 2001). Currently, the only available pure anabolic drugs are parathyroid hormone mimetics. The use of fully human monoclonal antibodies has also been approved for osteoporosis and a number of drugs are being tested clinically for osteoporotic treatment and prevention (Lewiecki, 2011; Padhi, Jang, Stouch, Fang, & Posvar, 2011). However, unsurprisingly, there is a lack of interventional studies regarding the treatment of osteoporosis in patients with COPD.

Antioxidant treatment for osteoporosis has proven to be quite effective. The beneficial effects of antioxidants with regards to bone health and osteoporosis have also been demonstrated epidemiologically and through clinical intervention (Rao, 2013). Bearing in mind the possible adverse effects of hormonal therapy and the increasing reports regarding the side effects of bisphosphonates in the management of osteoporosis, there is a high demand for complementary and/or alternative medicine for the prevention and treatment of osteoporosis. This is especially true for COPD patients who are inherently at a greater risk of developing the disease. Due to the damaging effects of ROS on bone growth and regulation, targeting oxidative stress in COPD patients may prove to be beneficial for both COPD and osteoporosis.

7. Novel antioxidant approaches for the treatment of chronic obstructive pulmonary disease

Besides never smoking, smoke cessation is the only effective method for preventing the onset and progression of COPD. The progression of airway inflammation, increased oxidative stress, and protease burden even months/year after cessation, and non-responsiveness to glucocorticosteroids have been documented as therapeutic challenges for the treatment of COPD (Kalyanaraman, 2013). Because of this, there is a lack of effective treatments for COPD and its comorbidities.

As stated throughout this review, tissue injury and inflammation as a result of oxidative stress are common to COPD and many of its comorbidities. A common theme throughout this review is the similarity and interplay between the pathological mechanisms of each condition and it is this similarity that provides a unique therapeutic opportunity (Fig. 6). Treatment with antioxidants may be able to progressively prevent and treat multiple diseases by suppressing the generation of ROS/RNS, neutralizing oxidants or both. Due to the overwhelming evidence implicating oxidative stress in the pathogenesis of COPD and its comorbidities, it is only logical to consider antioxidant intervention in this patient population. It is important that such interventions not only neutralize the excessive ROS and RNS generated, inhibit peroxidation of lipids, and the subsequent inflammatory response, but to also identify the source of these oxidants and inhibit their generation (Selemidis et al., 2008). Due to the imbalance in oxidants and antioxidants, this can be achieved by two approaches: increasing the endogenous antioxidant enzyme activity via enzyme modulators/mimetics, or by replenishing the depleted non-enzymatic defences through dietary or pharmacological means.

It should be noted that typical radical scavenging treatments, such as vitamin E and other dietary antioxidants, have shown minimal improvements in either COPD or the comorbidities outlined in this review. This may be due to the dose, route of administration or the specific antioxidant given and thus more research is required to determine the efficacy of these compounds with certainty. In addition, traditional antioxidants act in a “sacrificial manner,” due to the fact that once they have scavenged a radical, they are essentially consumed.

Undoubtedly, the most damaging of all the ROS/RNS are the hydroxyl radical and peroxynitrite. These species are extremely reactive and
indiscriminate to the point that they basically react with the first substrate they come into contact with. Knowing this, the most effective form of protection would be to prevent their generation in the first place and this cannot be performed by traditional antioxidant scavengers. Therefore, a pharmacological approach which inhibits the production of oxidant species is required. In addition to this, it may also prove beneficial to use combination therapy and treat patients with the pharmacological enzymatic antioxidants (e.g., NOX inhibitors) whilst also supplementing them with dietary antioxidants (e.g., vitamin E supplements).

7.1. Novel antioxidants

As shown on the ROS cascade (Fig. 2), there are 3 key enzymes that can be targeted in order to reduce oxidative stress; NOX, SOD, and Gpx/Cat. These enzymes can either be inhibited to reduce their activity (e.g., NOX inhibitor — apocynin) or amplified/mimicked to increase their activity (e.g., Gpx mimetics — ebselen). Outlined below are just some of the enzymatic compounds that may be of therapeutic benefit to COPD patients (also see Table 1).

7.1.1. NADPH oxidase inhibitors

As stated earlier, NOX-1 and -2 are the primary generators of O$_2^-$ and are responsible for the initiation of the ROS cascade (Fig. 2). NOX is a unique target in the sense that inhibiting the activity of the enzyme would not only reduce the generation of a single oxidant species but also reduce the generation of all other ROS and RNS. Inhibiting NOX would reduce O$_2^-$ production which would result in less available O$_2^-$ for the generation of H$_2$O$_2$ and ONOO$^-$, subsequently reducing OH generation and increasing NO bioavailability as a result.

7.1.1.1. Current studies. There are several NOX inhibitors currently being studied, however the most common is apocynin, which is a NOX inhibitor that preferentially blocks NOX-2 at low doses. It inhibits NOX by preventing the assembly of the NOX enzyme subunits, resulting in the reduced formation of NOX complexes (Sellemidis et al., 2008; Drummond et al., 2011). We have shown that apocynin reduces cigarette smoke-induced lung inflammation in mice (Bernardo et al., unpublished observations). Additionally, the inhibition of NOX-2 activity ameliorates influenza A virus-induced lung inflammation, indicating that pharmacologically targeting NOX-2 may also have therapeutic potential in seasonal and possibly pandemic influenza infection (Vlahos et al., 2011; Vlahos, Stambas, & Selemidis, 2012). Because of this, the possible therapeutic utility of NOX-2 inhibitors may extend to AECOPD. Clinically, COPD patients treated with apocynin had reduced H$_2$O$_2$ and NO$_2^-$ in their exhaled breath concentrate compared to placebo control (Stefanska et al., 2012).

7.1.2. Superoxide dismutase mimetics

There are 3 human isoforms of SOD (1, 2, and 3), each of which can transform O$_2^-$ to H$_2$O$_2$. SOD3 (or extracellular SOD) is located in the extracellular matrix, the junctions of airway epithelial cells, the surface of airway smooth muscle, and the lining of blood vessels of the lung (Kinnula & Crapo, 2003). It should be noted that smokers and COPD patients have increased spumum levels of SOD3 as an adaptive response to the increased oxidative burden (Regan et al., 2011). Additionally, polymorphisms in the SOD3 gene have been associated with emphysema but not COPD susceptibility (Sorheim et al., 2010). It is possible to enhance the conversion of O$_2^-$ to H$_2$O$_2$ by introducing SOD mimetics. This would result in the same effects caused by NOX inhibition: reduced O$_2^-$, increased NO bioavailability, however, it would also increase H$_2$O$_2$ generated. Although there is increased H$_2$O$_2$ generation, the newest class of SOD mimetics (salen complexes — “salens”) have also shown Catalase like activity, enhancing the neutralization of H$_2$O$_2$ in cells and decomposing ONOO$^-$ (Sharpe, Olsson, Stewart, & Clark, 2002). Additionally, it has been suggested that SOD mimetics may be beneficial for the treatment of cancer in combination with traditional cancer therapies (Thomas & Sharifi, 2012).

7.1.2.1. Current studies. Since the administration of exogenous SODs themselves has often proven to be problematic, a variety of innovative approaches are currently being explored, one of these being SOD mimetics. Multiple classes of SOD mimetics have been developed and each class has generally been effective in animal models of COPD. Administration of the SOD mimetic M40419 in rats treated with VEGF receptor blockers significantly decreased markers of oxidative stress in the lungs and prevented the development of emphysema (Tuder et al., 2003). In cigarette smoke-exposed rats, treatment with the SOD mimetic AEOL 10150 was found to significantly reduce BALF inflammation (Smith et al., 2002). It is known that the acute loss of SOD3 in adult mice causes death, whereas overexpression of SOD3 in animals exposed to hyperoxia reduces mortality (Folz, Abushamah, & Suliman, 1999; Gongora et al., 2008). Additionally, administration of SOD mimetic significantly attenuated the elastase-induced emphysema in both wild-type and SOD3 knockout mice (Yao et al., 2010). This study found that SOD3 protected against oxidative fragmentation of extracellular matrix (ECM), resulting in the mitigation of lung inflammatory response and emphysema. Knowing this, it is possible that SOD3 may be more effective than other SOD isoforms for the management of COPD. Therefore, the development of pharmacological mimetics to replenish and augment SOD3 in the lung may have a therapeutic potential for the treatment of COPD/emphysema (Rahman, 2012).

7.1.3. Glutathione peroxidase mimetics

The Gpx family of enzymes, along with catalase, are responsible for the termination of the ROS cascade. There are 8 known isoforms of Gpx (1 to 8) but the most abundant isoform is Gpx-1, which is found in the cytoplasm of almost all mammalian cells and whose preferred substrate is H$_2$O$_2$ (Vlahos & Bozinovski, 2013). The main function of Gpx is the reduction of H$_2$O$_2$ to H$_2$O and O$_2$; however, Gpx is also known to reduce lipid peroxides to their corresponding alcohols. Additionally, it has been reported that Gpx-2 is a major cigarette smoke-inducible isoform found in the lung (Singh et al., 2006). As with SOD,
it is possible to increase the reduction of H2O2 by introducing Gpx (or catalase) mimetics. This would result in the termination of the ROS cascade by increasing conversion of H2O2 to H2O and O2, and subsequently reduced levels of •OH and decreased levels of lipid peroxides.

7.1.3.1. Current studies. There is currently a limited amount of studies exploring the effectiveness of Gpx mimetics in COPD. When exposed to cigarette smoke, Gpx-1 knockout mice exhibited increased BALF neutrophils, macrophages, proteolytic burden, whole lung IL-17A, and MIP1 mRNA compared with WT mice (Duong et al., 2010). When treated prophylactically with the Gpx-1 mimetic ebselen, the cigarette smoke-induced increases in BALF macrophages, neutrophils, proteolytic burden, and macrophage and neutrophil chemotactic factor gene expression were all inhibited in both the WT and knockout mice. In addition, ebselen inhibited established BALF inflammation when administered therapeutically, suggesting that Gpx-1 mimetics may have therapeutic utility in inflammatory lung diseases where cigarette smoke plays a role such as COPD (Vlahos & Bozinovski, 2013). In addition, we have shown that ebselen caused a reduction in influenza A virus-induced lung inflammation in mice, suggesting that targeting Gpx-1 may be of therapeutic benefit for AECOPD (Yatmaz et al., 2013).

Ebselen has also been shown to be protective in vivo in disease situations hallmarkmed by oxidative stress such as diabetes-associated atherosclerosis and cerebral ischaemia–reperfusion injury (Wong, Bozinovski, Hertzog, Hickey, & Crack, 2008). Additionally, ebselen has been used in clinical trials of acute ischaemic stroke and was found to improve the outcome of patients when administered within 24 h of stroke (Yamaguchi et al., 1998).

7.1.4. Nuclear factor erythroid 2-related factor 2 activators

In addition to the 3 enzymes in the ROS cascade, the DNA binding protein nuclear factor erythroid 2-related factor 2 (Nrf2) presents a potential target for reducing oxidative stress in COPD (Boutten, Goven, Artaud-Macari, Boczkowski, & Bonay, 2011). Nrf2 is found in the cytoplasm of many mammalian cells and is responsible for the regulation of various antioxidants and cytoprotective genes, acting as a “master switch” for these genes. In response to oxidative stress, Nrf2 translocates to the nucleus and binds to the antioxidant response element (ARE) of target genes, along with other binding factors and cofactors, resulting in the induction of stress response genes (Rahman, 2012). COPD patients have decreased levels of Nrf2 in the lungs, and may be linked to the reduced antioxidant capacity observed in patients (Nguyen, Nioi, & Pickett, 2009). In addition, Nrf2 may also help improve the clearance of bacteria by alveolar macrophages, which is particularly relevant to the prevention and treatment of AECOPD (Harvey et al., 2011).

7.1.4.1. Current studies. It is known that Nrf2 signalling is impaired in several chronic diseases, including COPD, cancer, and neurodegenerative diseases (Boutten et al., 2011; Bauer, Hill, & Alexander, 2013). The levels of Nrf2 protein are decreased in lungs of patients with COPD and one study found that Nrf2-dependent antioxidants was negatively associated with severity of COPD, suggesting that therapy directed towards enhancing Nrf2-regulated antioxidants may be a novel strategy for attenuating the effects of oxidative stress in the pathogenesis of COPD (Malhotra et al., 2008). Studies have shown increased susceptibility of Nrf2 knockout mice to CS- or elastase-induced pulmonary emphysema (Rangasamy et al., 2004; Iizuka et al., 2005; Ishii et al., 2005). Compared with WT littermates, the Nrf2 knockout exhibited more pronounced inflammation and neutrophilic elastase activity in the BALF, enhanced alveolar expression of oxidative stress markers, increased numbers of apoptotic endothelial and alveolar type II epithelial cells, and decreased antioxidant and antiprotease gene expression in alveolar macrophages. Nrf2 deficiency also results in impaired alveolar type II cell growth and enhanced sensitivity to oxidants, thereby contributing to abnormal lung injury and repair (Boutten et al., 2011). Treatment with the Nrf2 activator CDDO-imidazolide was found to attenuate cigarette smoke-induced emphysema and cardiac dysfunction in mice (Sussan et al., 2009). Nrf2 appears to protect against pulmonary hyperoxia/bleomycin injury and ovalbumin challenge in mice, presumably by upregulating the transcription of lung antioxidant defence enzymes. It was also found that treatment with Nrf2 activators may be able to restore glucocorticosteroid sensitivity in COPD patients (Malhotra et al., 2011). A clinical trial of the Nrf2 activators sulforaphane is currently in progress in patients with COPD (Barnes, 2013).

Activation of Nrf2 has also been observed to have anticancer effects. Several studies have found that Nrf2-activating compounds can prevent or suppress cancer in mouse models (Sporn & Liby, 2012).

8. Concluding remarks

COPD is a major incurable global health burden and is the 4th largest cause of death in the world. Exacerbations are a common occurrence in COPD patients and contribute mainly to morbidity, death and health-related quality of life. Comorbid diseases, in particular cardiovascular disease and skeletal muscle wasting, are also common in COPD and potentiate the morbidity of COPD, leading to increased hospitalisations, mortality and healthcare costs. Current treatments have limited efficacy and fail to modify the factors that initiate and drive the long-term progression of COPD, its exacerbations and its co-morbidities. In addition, no pharmacological treatment has been shown to reduce the risk of death in COPD in prospective clinical trials. It is now evident that increased oxidative stress within the local lung microenvironment is a major driving mechanism in the pathophysiology of COPD and that it may directly influence peripheral organ (e.g., heart, skeletal muscle, brain, bone) behaviour in a ‘COPD-specific manner’. Therefore, targeting oxidant-dependent mechanisms that drive COPD and its co-morbidities may have great therapeutic potential.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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