The Role of Oxidative Stress in Smoking-Related Diseases[#]

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Abstract: Oxidative stress, the accumulation of oxygen free radicals (reactive oxygen species) above and beyond the capacity of a cell to utilise antioxidant systems to detoxify these potentially damaging molecules, is a common feature of many human disorders. Cigarette smoke is not only a source of free radicals but is also a potent stimulator of the intracellular production of free radicals, by the mitochondrial electron transport chain and the plasmalemmal NADPH oxidase. Adding to this free radical burden is the reduction, by cigarette smoke, of the cellular antioxidant capacity. Together, the increased production and reduced detoxification of free radicals has been strongly linked to smoking-induced diseases including atherosclerotic cardiovascular disease, cancer and chronic obstructive pulmonary disease (COPD). In this review, we discuss the mechanisms underlying cellular free radical production, relate this to the three major smoking-related human diseases listed above and present potential mechanisms by which cigarette smoke may increase the oxidative burden on cells and contribute to disease.

Keywords: Reactive oxygen species, antioxidants, cardiovascular disease, atherosclerosis, cancer, chronic obstructive pulmonary disease, COPD, cigarette smoke.

[#]This paper is dedicated to the memory of Dr Stephen P. Faux, who believed that nothing is more exciting and entertaining than science. He is much missed as a friend, mentor and colleague.

INTRODUCTION

Within functioning biological systems, cells are continuously exposed to oxidants which include oxygen free radicals, termed reactive oxygen species (ROS). These oxidants arise from a number of sources including aerobic respiration within the mitochondrial electron-transport chain [1], by the action of the antimicrobial defence mechanisms in neutrophils and macrophages which utilise NADPH oxidase to generate the superoxide anion [2], or are derived from free radicals or pathogens in the external environment. During normal physiological function there exists a balance between antioxidants and oxidants and under normal circumstances oxidants are removed by intracellular antioxidant defence systems which include, in particular, glutathione and thioredoxin [3]. However, if there is an increase in oxidants or a decrease in antioxidants this balance becomes disrupted leading to oxidative stress [4]. If left unregulated, excess oxidants can react with cellular components resulting in injury within the cell, tissue or organ [1-4].

Cigarette smoking is a cause of serious and fatal diseases including cardiovascular disease, cancer and chronic obstructive pulmonary disease (COPD). Cigarette smoke is a complex and dynamic mixture of more than 5,300 individual chemical constituents [5] and there is strong evidence linking cigarette smoking with a number of disease processes. Cigarette smoke is known to be both a source and an inducer of cellular oxidative stress, which is a factor in many smoking-related diseases [2, 6-8] and this oxidative stress initiates a variety of pathological processes which contribute to disease development.

Broadly speaking, there are two potential sources of cigarette smoke-induced oxidative stress. Firstly, both the particulate and gas phases of the cigarette smoke are direct and rich sources of exogenous free radicals of many different species. These radicals fall into a number of different categories including both short- and long-lived species [9, 10]. An in-depth review of the chemical nature of these radicals can be found in Wooten *et al.* [11]. Secondly, it is becoming increasingly recognised that cigarette smoke can induce the production of oxidative species by many of the cellular enzyme systems described later in this chapter. Induction of oxidative stress in this manner may be exacerbated by cigarette smoke concomitantly decreasing the protection afforded by antioxidants such as vitamin C (ascorbic acid), carotene, glutathione peroxidase and superoxide dismutase [12-14]. Whatever the cause of the smoke-induced oxidative stress, there are large bodies of evidence linking cigarette smoke with the induction of cellular oxidative stress and the initiation and progression of each of the three major smoking-related diseases mentioned above. In this review, we will provide a brief overview of each of these diseases, and subsequently describe some of the evidence linking disease processes with cigarette smoke-induced oxidative stress.

CARDIOVASCULAR DISEASE

Cardiovascular disease is the major cause of mortality in the Western world. American Heart Association figures have shown that, in the USA alone, more than 80 million people suffer from some form of cardiovascular disease. In 2004, this led to the loss of around 870,000 lives and a large proportion of these deaths occurred before average life expectancy [15]. Cardiovascular disease manifests itself in many forms but may be broadly classified into coronary heart disease, cerebrovascular disease and peripheral vascular disease. The common residing feature within each of these classifications is compromised blood supply to tissues and organs, reducing the delivery of oxygen and nutrients to respiring cells and inducing pathogenic changes in cell function. The fundamental basis of cardiovascular disease is the formation of an atherosclerotic plaque or lesion, which is often also termed an atheroma. This is, in essence, a thickening of the blood vessel wall which can occlude the lumens of blood vessels and disrupt blood flow, leading to both acute and chronic manifestations such as heart attacks and strokes. The likelihood of an individual developing cardiovascular disease is modulated by a number of risk factors, which fall into two categories: modifiable and fixed. The modifiable factors are a series of environmental cues and lifestyle choices including diet, smoking, concurrent disease status (e.g. diabetes), alcohol consumption and exercise [16]. Fixed risk factors include genetic composition, age, menopausal status, and gender. Importantly, the role that many of these risk factors play in cardiovascular disease initiation and development may be linked to oxidative stress, both by increasing free radical production and by down-regulating key antioxidant systems [12, 14, 17-23]. This commonality of the link between oxidative stress, the various risk factors and disease likelihood perhaps highlights the importance of oxidative stress in cardiovascular disease.

Atherosclerosis was initially considered to be a disease simply involving accumulation of lipids within arteries. However, it is now clear that it involves a rather more complex cascade of inflamma-

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tory processes [24-26]. An initiating step in atherosclerosis development is damage to the endothelium [27], a monolayer of cells lining blood vessels which regulates many facets of vascular function. In a healthy individual and prior to cardiovascular disease onset the endothelium has a role in regulating vascular tone and blood flow, thus maintaining vessel homeostasis [27]. When the endothelium becomes damaged and dysfunctional a chronic inflammatory process is triggered in the vessel itself. This process eventually involves a host of different cell types within the cardiovascular system [2], all of which contribute to atherothrombus formation and disease progression. In brief, damaged endothelial cells express surface markers (adhesion molecules) which attract circulating monocytes, tethering them to the endothelial surface and facilitating their transmigration into the subendothelial space. Once there, these monocytes differentiate into tissue macrophages and begin secreting inflammatory molecules such as cytokines which further aid in plaque development. Macrophages take up circulating oxidised lipids such as the low density lipoprotein and become lipid-laden foam cells, giving rise to the fatty streak which is a hallmark of atherosclerosis. Vascular smooth muscle cells also migrate into the tissue layer just beneath the endothelium where they proliferate, at least in part due to cytokine stimulation, and promote the growth of the advancing plaque into the vessel lumen. Platelets are also recruited to the growing lesion, eventually forming a fibrous cap which can detach and cause a vessel blockage elsewhere in the cardiovascular system. For each of the cellular processes described above oxidative stress has been implicated as a supporting factor.

THE INVOLVEMENT OF OXIDATIVE STRESS IN CARDIOVASCULAR DISEASE PROCESSES

The literature implicating oxidative stress in cardiovascular disease is vast and growing, and a full and complete discussion is perhaps outside of the scope of this review article. This literature has been reviewed in detail elsewhere [e.g. 2, 28-30]. However, in the following section we highlight some of the key points concerning oxidative stress and cardiovascular disease and in particular emphasise the key role of intracellular sources of free radicals in disease development.

The mitochondrial electron transport chain plays an important role in cell function by producing energy in the form of adenosine-5'-triphosphate (ATP) by oxidative phosphorylation using oxygen and glucose as substrates. The mitochondrial electron transport chain consists of a series of enzymatic complexes, the major function of which is to generate a proton gradient across the inner mitochondrial membrane and thus provide a proton-motive force to drive ATP production by the enzyme ATP synthase. Although this process is efficient, a proportion of electrons may be lost from electron transport chain complexes [31], predominantly from complexes I and III. These electrons interact with oxygen to produce ROS such as the superoxide (O_2^-) anion [32, 33], thus providing a potential source of oxidative stress. In pathologic states, and for example in those individuals exhibiting some of the proatherogenic risk factors described above, electron transport chain function can be modified to give rise to increased ROS production [34, 35]. While ROS production *per se* is not necessarily damaging it is the accumulation of these ROS, due to both over production and a lack of detoxifying antioxidant systems, which leads to oxidative stress and disease propensity by causing damage to cellular DNA, proteins and lipids.

Numerous lines of evidence have also suggested that a major source of ROS in cardiovascular disease is the enzyme complex, NADPH oxidase. This cell membrane-associated complex was first discovered in the immune system where a high level of ROS production in phagocytes mediates the killing of ingested pathogens such as bacteria [36]. In essence, this enzyme complex generates a large burst of superoxide (the 'oxidative burst') on the extracellular side of the membrane by the one electron reduction of oxygen, using NADPH as an electron donor [37, 38]. NADPH-dependent ROS-generating activity has also been documented in numerous non-immune system cells in the cardiovascular system, including vascular smooth muscle cells, endothelial cells, fibroblasts and cardiac muscle cells. The major evidence linking NADPH oxidase to cardiovascular disease has come from experimental models of vascular disease, for example in models examining cholesterol-induced atherosclerosis [39] or genetic disruption of the oxidase [40]. It is also of note that, in humans, genetic changes in NADPH oxidase expression and/or function may contribute to atherosclerosis susceptibility [41, 42], while increased O_2^- generation by NADPH oxidase in vessels has been linked to atherosclerosis risk factors [43].

While mitochondria and NADPH oxidase are major ROS sources which contribute to atherogenesis, they perhaps do not alone account for all ROS produced and other cell enzyme systems may also provide a source of oxidative stress. For example, ROS may be produced by enzymes such as xanthine oxidase, nitric oxide synthase, lipoxygenase and myeloperoxidase. It is also worthy of mention that down-regulation of antioxidant systems, such as glutathione peroxidase, heme oxygenase and superoxide dismutase have all been proposed to contribute to oxidative stress [2].

The sources of oxidative stress described above are plentiful and play key roles in cardiovascular disease development. Importantly, for each of the cell types and individual cellular processes involved in atherogenesis there is also a wealth of literature linking oxidative stress with altered cellular function. In endothelial cells, vascular smooth muscle cells, monocytes, macrophages and platelets, oxidative stress drives key changes in atherogenic processes which ultimately lead to lesion formation. To pick just a single example, the expression of adhesion molecules on the cell surface of monocytes, endothelial cells and platelets may be induced by oxidative stress [44-46]. For almost every other atherogenic process similar literature exists and while the finer details of which oxidant and antioxidant systems are involved in which processes and in which cell types may be debatable, what is very clear is the generic role of oxidative stress in cardiovascular disease.

CIGARETTE SMOKE, OXIDATIVE STRESS AND CARDIOVASCULAR DISEASE

There is strong evidence linking cigarette smoking with alterations in a number of cardiovascular disease processes. Underpinning many of these changes is cellular oxidative stress, a phenomenon at least partly causative of cardiovascular disease, not only due to smoking but also due to many other risk factors including diabetes mellitus, excessive alcohol consumption, hypercholesterolaemia (elevated blood lipids) and aging [2]. While both exogenous (radicals found in cigarette smoke) and endogenous (intracellular radical production induced by cigarette smoke or its components) oxidants are likely to contribute to oxidative stress and cardiovascular disease progression, we will focus here on a biologist's perspective of induced free radicals and altered disease processes.

As mentioned above, the vascular endothelium plays a pivotal role in maintaining the homeostasis of the cardiovascular system by controlling thrombosis and thrombolysis, platelet and leukocyte interactions with the vessel wall, and by regulating vessel tone by secreting vasorelaxants such as nitric oxide and vasoconstrictors such as endothelin-1 [47, 48]. Endothelial damage and dysfunction therefore reduces the efficacy of these functions, providing an initiating stimulus for pro-atherosclerotic events in the vessel. Many studies have examined and characterised the role of cigarette smoke and its constituents in damaging the endothelium. For example, Vayssier-Taussat *et al.* [49] demonstrated that exposure of cultured endothelial cells to cigarette smoke extracts produced by passing

cigarette smoke through an aqueous buffer solution altered various aspects of endothelial function. These effects were prevented by the antioxidant *N*-acetylcysteine, demonstrating the role of oxidants in the response to smoke exposure. The authors further studied how the endothelial cells responded to exposure to the cigarette smoke toxicant benzo(a)pyrene, and concluded that the effects of cigarette smoke were independent of the benzo(a)pyrene content of the smoke [49]. In a more recent comprehensive study [50], electron spin resonance was used to demonstrate that superoxide anions were produced both intracellularly and in the extracellular space in response to aqueous extracts of cigarette smoke. This exposure also lead to the production of the peroxynitrite ($ONOO^-$) anion, a potent reactive oxygen species strongly implicated in vascular damage. The formation of both superoxide and peroxynitrite were reduced by the antioxidants ascorbate and α-tocopherol, and since studies using selective inhibitors ruled out an involvement of NADPH oxidase in superoxide production the authors hypothesized that redox-active quinines present in the aqueous extracts are responsible for superoxide production [50]. They did however concede that mitochondria may also provide a source of superoxide, given the subsequent findings of Csiszar *et al.* [51] who demonstrated that cigarette smoke extracts increased the fluorescence of cells loaded with a dye which specifically detects mitochondrial superoxide, an effect which was reversible with the antioxidant resveratrol.

While endothelial injury itself initiates atherosclerosis, endothelial repair by the processes of migration and proliferation re-establish endothelial integrity and are atheroprotective [24, 52-54]. In 2001, Snajdar *et al.* [52] demonstrated the inhibitory effects of tobacco smoke particulate extracts on the migration of cultured endothelial cells. Given that reactive oxygen species regulate endothelial migration [55], it has recently been shown that the exposure of endothelial cells to aqueous cigarette smoke extracts inhibited endothelial migration in an oxidative stress-dependent manner since the effects of the extracts were reversed by the antioxidants *N*-acetylcysteine or ascorbate [56]. Interestingly however, our own unpublished data suggest that when exposing cells to the particulate phase only of the cigarette smoke, impairment of migration was *independent* of induced oxidative stress. This leads to the possibility that different phases of cigarette smoke exert inhibitory effects on endothelial function via different mechanisms, both including and excluding oxidative stress.

Monocytes play a key role in atherosclerotic lesion formation. These circulating cells attach to the damaged endothelium, prior to migration through the endothelial monolayer into the subendothelial space, where they differentiate into macrophages [25]. Here, macrophages take up lipids, beginning the process by which the growing plaque becomes lipid-laden. Oxidative modification of these lipids enables their uptake into macrophages, and cigarette smoke has been described to facilitate this modification *in vitro* in a manner dependent on superoxide production [e.g. 57]. Furthermore, smoking increases lipid modification *in vivo* and in smokers, oxidised lipid levels are markedly increased [58]. Monocytes/macrophages also secrete inflammatory agents such as interleukin-8 (IL-8) which contribute to vessel damage and inflammation in atherosclerosis. Facchinetti *et al.* [59] demonstrated this process to involve ROS production, a finding more recently replicated by others [60]. A key step in transducing cigarette smoke exposure into a pro-inflammatory signal is the activation of oxidative stress-responsive transcription factors such as NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and Nrf2 (nuclear factor erythroid-2-related factor 2) and there is a host of evidence, not only in monocytes but also in endothelial and other cell types, of cigarette smoke-induced NF-κB and Nrf2 activation secondary to free radical production [61, 62].

Given the number of potential intracellular sources of ROS the question arises, which of these may be regulated by cigarette smoke and contribute to the oxidative stress burden? The superoxide-producing enzyme complex NADPH oxidase may play a role, since in many cardiovascular cells cigarette smoke extracts activate this enzyme and produce superoxide [61, 63]. Conflicting data however suggest a lack of a role for this oxidase [64] but this may be related to differences in the origin of the endothelial cells used in these studies or to the different endpoints examined. The mitochondrial electron transport chain, a source of free radicals during physiological cellular respiration but which is also stimulatable under pathological conditions, may also contribute to oxidative stress in response to cigarette smoke. For example, treatment of endothelial cells and monocytes with smoke extracts induced a loss of the mitochondrial membrane potential, indicative of mitochondrial dysfunction [49, 65]. In the study by Vayssier-Taussat this loss of mitochondrial function was followed by cell death due to apoptosis or necrosis in both endothelial cells and monocytes, an effect which was prevented with the antioxidant, *N*-acetylcysteine. These studies are in agreement with a more recent study in which cigarette smoke induced mitochondrial ROS production, transcription factor activation, upregulation of inflammatory markers, DNA damage and apoptosis in endothelial cells [51]. These effects were prevented by the antioxidant resveratrol. Similar findings have been reported in lung epithelial cells [66]. The role of the mitochondria is also further supported by historical studies in isolated mitochondria, in which cigarette smoke extracts altered *in vitro* mitochondrial respiration [67, 68] and by more recent *in vivo* studies in atherosclerosis-susceptible mice exposed to secondhand cigarette smoke [69].

The endothelial isoform of nitric oxide synthase (eNOS), an enzyme responsible for producing the vasorelaxant nitric oxide which is important in controlling vascular tone, has become a centre of attention due to its potential role in cigarette smoke-induced superoxide production. Under certain conditions eNOS becomes uncoupled from a NO to a O_2^- producing state [70]. The O_2^- produced can interact with NO to produce $ONOO^-$, a damaging and cytotoxic free radical with the potential to disturb cardiovascular function itself and also to form other secondary radicals [50]. $ONOO^-$ itself reduces the bioavailable levels of NO leading to a reduction in endothelial vasoregulatory capacity and increasing vascular dysfunction. $ONOO^-$ also inactivates BH_4, an eNOS cofactor, effectively amplifying the damaging effects of eNOS uncoupling on the endothelium [71]. Aqueous cigarette smoke extracts lead to the production of $ONOO^-$ in endothelial cells [50]. Numerous studies have examined the levels of cellular expression of eNOS in response to endothelial cell exposure, and both decreases and increases in expression have been reported [58, 71, 72]. Cigarette smoke extracts may also directly alter eNOS function [50]. These apparently contradicting reports demonstrate a need for clarification of the underlying mechanisms and responses. However, it is clear that altered NO levels, the ensuing alterations in bioavailability of this controller of vascular function and the potential for increases in damaging radicals all contribute to oxidative stress and cardiovascular disease. In support of this notion are studies demonstrating that free radical scavengers improve NO production and eNOS activity [73].

Of the many known chemicals found in cigarette smoke, surprisingly little is known concerning the effects of any potential individual toxicants with respect to cardiovascular disease. However, some attention has been paid to acrolein, a chemical constituent of the vapour phase of cigarette smoke which has been shown to induce free radical production in endothelial cells [74, 75] and macrophages [59], in the latter case leading to the activation of enzymes responsible for destabilising atherosclerotic plaques which lead to acute cardiovascular events [76]. Many potential sources of ROS are implicated in these responses, including inactivation of the antioxidant thioredoxin reductase [77] and activation of xanthine oxidase (but not mitochondria or NADPH oxidase) [78]. Acrolein has been further shown to cause oxidative damage and mitochondrial dysfunction, although this was in retinal epithelial cells and not the cardiovascular system [78]. Intriguingly, acrolein may itself

initiate protective responses subsequent to its ability to induce an oxidative insult and has been shown to induce the expression of the antioxidants thioredoxin reductase [77] and heme oxygenase-1 [74] in endothelial cells. Despite these few studies and given the current lack of detailed knowledge of toxicant behaviour, further studies are clearly required to give insight into the role of individual smoke toxicants in inducing cellular oxidative stress and cardiovascular disease.

A final note on the role of the particulate phase of cigarette smoke in cardiovascular disease development. A recent interesting study utilising cardiovascular disease-susceptible mice has demonstrated that inhalation of concentrated ambient particulate matter (CAPs) of less than 2.5 μm in diameter for 4 months enhanced the expression of the $p47^{phox}$ and rac1 components of the vascular NADPH oxidase and induced vascular superoxide production [79]. Both of these effects were associated with an enhanced atherosclerotic burden, detected as an increased plaque area in the thoracic aorta of the animals. Furthermore, human volunteers exposed to diesel exhaust particles demonstrated impaired vascular function, and altered nitric oxide bioavailability secondary to oxidative stress has been proposed to underlie this response [80]. Supporting studies have been performed *in vitro*, albeit in lung epithelial cells, in which particulate matter exposure generated oxidants via complex III of the mitochondrial electron transport chain [81]. These studies highlight the need, when attempting to elucidate the mechanisms of smoke-induced oxidative stress, to examine both the gas and particulate phases of cigarette smoke extracts, particularly since *in vivo* studies have demonstrated the ability of particulate matter to enter the cardiovascular system and exert an oxidative stress burden.

CANCER

Cancer is defined as the uncontrolled growth of abnormal cells in the body. This can lead to serious adverse effects on the host through invasive growth and metastasis (spreading of cancer from original site to distant locations in the body via the bloodstream and the lymphatic system). Cancer is a leading cause of death worldwide. From a total of 61 million deaths worldwide in 2007, cancer accounted for 7.9 million (or 13%) of the total. In the UK approximately 293,000 new cases were diagnosed in 2006 [82], while 155,484 people died from cancer in 2007 [83]. Lung cancer accounted for the largest proportion (22%) of all cancer deaths in the UK in 2007 [83], and is also the most prevalent cancer type responsible for mortality globally, causing 1.4 million deaths in 2007 [84].

Notwithstanding its complex nature, cancer can be viewed in terms of a few underlying principles. It has been proposed that, despite the numerous cancer genotypes there are six physiological traits that most, if not all, cancers have in common [85]. These traits include the ability of cancer cells to generate their own mitogenic signals, to become resistant to exogenous growth-inhibitory signals, to become resistant to programmed cell death (evasion of apoptosis), to divide limitlessly (immortalisation), to acquire vasculature (angiogenesis) and eventually to invade and metastasise. There is much evidence to suggest that the first five traits may be both necessary and sufficient for the development of primary tumours in man, and many of the genetic elements of these pathways have been established [86-89]. The processes underpinning the final steps in tumour progression (invasion and metastasis) have remained more elusive. Recent discoveries in this area of research, such as the role of the epithelial-mesenchymal transition program in metastasis, have made some progress in addressing this situation [90, 91].

The multi-stage process of carcinogenesis can be divided into initiation (mutation of cells), promotion (selection and clonal expansion of mutated cells) and progression (irreversible acquisition of malignant potential). Among the genetic events that drive cells towards a malignant state are mutations that increase expression of oncogenes, or decrease the activity of tumour suppressor genes. An oncogene is any gene that encodes a protein which can transform cells in culture or induce cancer *in vivo*. Most of these are activated forms of normal cellular genes (proto-oncogenes) involved in cell growth, proliferation, survival and differentiation [92]. Tumour suppressor genes, on the other hand, are guardians against DNA damage induced by chemicals, irradiation or an excess of proliferative signals [92]. The proteins encoded by tumour suppressor genes act to inhibit cell proliferation in one way or another. Mutations in both oncogenes and tumour suppressor genes can confer a growth or survival advantage on the cell (e.g. one or several of the six traits listed above) which culminate in tumour formation and/or progression.

THE INVOLVEMENT OF OXIDATIVE STRESS IN CARCINOGENESIS

Oxidative stress is thought to play a part in the initiation, promotion and progression phases of cancer [93, 94] and the role of oxidative stress in each of these phases is complex. ROS and also reactive nitrogen species (RNS) can act either directly by damaging DNA, lipids and proteins or indirectly through the recruitment of inflammatory mediators that trigger a secondary response. The following section illustrates how these interactions occur and their potential consequences in carcinogenesis.

High levels of ROS can cause extensive DNA damage that leads to genomic instability, thereby contributing to carcinogenesis. Endogenously-formed ROS such as the hydroxyl radical ($HO^{\cdot}$), which is generated during oxidative respiration, can cause alterations in purines and pyrimidines (DNA bases) which can in turn affect gene integrity. Any oxidative lesion that is not repaired can lead to mutation, increasing the risk of carcinogenesis [95]. An example of this is 8-hydroxy-2'-deoxyguanosine (8-OHdG), one of the most abundant and readily-formed oxidised DNA bases to have been identified in nuclear DNA. The types of mutations caused by 8-OHdG are associated with initiation, promotion and progression of tumours [95, 96], and increased levels of 8-OHdG during oxidative stress have been linked to carcinogenesis *in vivo* [97]. For example, higher levels of 8-OHdG have been detected in cancer tissue and in the blood of breast cancer patients [98]. While all four DNA bases are modified by ROS, GC base pair modifications lead to mutations much more frequently than those of AT [99]. G to T transversion mutations are the most frequent mutation in the human p53 tumour suppressor gene [100]. p53 plays a central role in orchestrating a number of anti-carcinogenic processes such as apoptosis and DNA repair. ROS-induced mutations that interfere with the proper functioning of p53 are therefore pro-carcinogenic [101].

RNS such as N_2O_3 can also cause mutations in DNA through the deamination of DNA bases to form mutagenic lesions. Deamination is a two-step process which involves the formation of a diazonium ion which is then hydrolysed. The net result of the replacement of an amino group with a hydroxyl group is the potential for the DNA bases to mispair, which can give rise to mutations. Some RNS can directly produce oxidative lesions in DNA and RNA, such as the formation of 8-nitroguanine (8-NG) by peroxynitrite ($ONOO^{-}$) [102]. 8-NG is an example of a DNA adduct (i.e. a piece of DNA which has been covalently bonded to a chemical). The stability of the 8-NG adduct in DNA is low but is much more stable in RNA [94]. 8-NG in RNA may interfere with RNA function and metabolism, thereby having a potential indirect effect on carcinogenesis [102]. Therefore RNS can be pro-mutagenic in a number of ways, depending on the free radical species involved.

In addition to mutations in nuclear DNA, mutations in mitochondrial DNA (mtDNA) are also found in tumours. However more evidence is needed in support of a causal role for mtDNA mutations in tumourigenesis [103]. A recent study to investigate this theory

involved the replacement of mtDNA from a poorly-metastatic mouse cell line with mtDNA from one that was highly metastatic. The recipient cells acquired the metastatic potential of the donor cells [104]. This could be reversed by the antioxidant *N*-acetylcysteine, indicating that ROS generation following mitochondrial mutation can increase metastasis [104].

Lipid peroxidation (the oxidative degradation of lipids) by ROS can also play a role in carcinogenesis. Lipid peroxidation is a three-step process involving the initial generation of reactive molecules such as $HO^{\cdot}$ and hydroperoxyl ($HO_2^{\cdot}$) radicals and the subsequent extraction of hydrogen from biomolecules. This is followed by radical chain propagation and termination. In abundance of O_2, this results in the generation of peroxyl radicals [105] which can form lipid hydroperoxides (LOOH). If these are not removed efficiently by glutathione (GSH)-dependent peroxidases they can degrade further in the presence of transition metals such as copper and iron to form lipid alkoxy ($LO^{\cdot}$), alkyl ($L^{\cdot}$) or $HO^{\cdot}$ radicals. This leads to another cycle of radical chain reactions yielding conjugated dienes and carbonyl compounds such as malondialdehyde (MDA), alkenals, alkadienals and α,β-unsaturated aldehydes such as crotonaldehyde and acrolein [106]. Some of these products can react with proteins and nucleic acids [107]. Many DNA adducts such as etheno-, propano- and MDA-DNA adducts can be formed from these products of lipid peroxidation [108]. The latter are mutagenic in mammalian and bacterial cells and carcinogenic in rats [94], and in humans such adducts have been identified in breast cancer tissue [109]. Increased levels of MDA-DNA adducts have also been found in other human cancers such as those of the larynx and colon, providing evidence of a link between oxidative stress and cancer through the formation of these DNA lesions [110, 111].

The oxidation of proteins may promote mutagenesis by damaging DNA polymerases (proteins involved in DNA replication) or inhibiting DNA repair mechanisms [112]. It is thought that the actions of ROS on intracellular signalling pathways through their modifying effects on protein phosphatases, protein kinases and transcription factors may play a more significant role in carcinogenesis than the more non-specific consequences of interactions with cellular macromolecules [113]. Redox-responsive cell signalling cascades for example can lead to increased cell growth in the presence of ROS.

The role of ROS in carcinogenesis, and the protective effect of antioxidant defences have been studied by using knockout mice, in which important antioxidant enzymes have been inactivated. For example, the inactivation of copper- and zinc-containing superoxide dismutase (CuZnSOD) resulted in increased liver cancer rates due to a resulting elimination of $O_2^{\cdot-}$ scavenging [114]. Manganese-containing superoxide dismutase (MnSOD) is currently thought to be a tumour suppressor gene. This is supported by evidence of reduced MnSOD levels in tumours of patients with colorectal cancer and the regression of these tumours when MnSOD expression is increased [115]. Tumour formation in a two-stage mouse model of skin carcinogenesis was also shown to be reduced following application of a phorbol ester to the initiated skin of mice in which MnSOD was overexpressed. In parallel, a reduction in the activity of activator protein-1 (AP-1), a transcription factor which regulates gene expression and is responsible for the control of a number of cellular processes including proliferation and apoptosis, was observed in these animals [116]. Conversely, a reduction of enzyme levels through knockout of the MnSOD gene resulted in increased AP-1 activity and increased production of oxidative damage proteins [117]. Given that AP-1 can play a central role in many human cancers [118], these animal studies highlight the role that ROS can play in carcinogenesis through transcription factor regulation.

At low levels ROS can trigger signalling cascades that can result in activation of transcription factors such as NF-κB and AP-1. This induces early response genes such as c-jun and c-fos which are involved in cell proliferation, angiogenesis, cell transformation and metastasis [119, 120]. The complex role of the NF-κB pathway in carcinogenesis has been reviewed extensively elsewhere [121].

In summary, free radicals are known to cause:

- Depletion of antioxidants and a decrease in antioxidant enzyme levels [94]
- Direct DNA damage and inhibition of DNA repair leading to mutagenesis [93, 120]
- Inhibition of apoptosis and induction of necrosis [94, 120]
- Activation of oncogenes and inactivation of tumour suppressors [120, 122]
- Stimulation of angiogenic factors [120]
- Amplification of inflammation by activation of cytokine production [120].

As a consequence of the impairment of these cellular processes, tumour formation is initiated or promoted. This emphasises the link between oxidative stress and carcinogenesis.

CIGARETTE SMOKE, OXIDATIVE STRESS AND CARCINOGENESIS

A number of studies have highlighted the central role of free radical-mediated processes in oxidative stress and tobacco smoke carcinogenesis [123-125]. For example, cigarette smoke tar has been found to contain a stable semiquinone radical that can reduce oxygen to superoxide and thus produce H_2O_2 and HO [126]. The particulate phase of cigarette smoke can also reduce levels of GSH in the lungs and thus leave the tissue susceptible to oxidative stress [127]. This can lead to damage to DNA and other biomolecules which has been supported by a number of studies in the literature [8, 128-130]. For example, 8-OH-dG was elevated in the lungs of mice treated with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific carcinogen [128]. A recent study in female A/J mice following whole-body exposure to cigarette smoke showed a significant increase in 8-OH-dG levels immediately after cessation of smoke exposure [129]. This was repaired over time. However, the authors proposed that the initial increase in this DNA lesion following smoke exposure may lead to mutations which could eventually lead to malignant transformation of these cells [129].

Nitric oxide (NO) is a constituent of cigarette smoke and can also be produced by the body through the action of nitric oxide synthase (NOS) [131]. NO has been implicated in tumour initiation, promotion and progression through direct damage to DNA and inhibition of DNA repair, enhancement of oncogene expression and angiogenesis and inhibition of apoptosis [132]. A study in rats exposed to the gas phase of cigarette smoke found increased expression levels of inducible nitric oxide synthase (iNOS), NF-κB, mitogen-activated protein kinases (MAPKs) and c-fos in the terminal bronchioles of the lungs. This led the authors to postulate that cigarette smoke induces oxidative stress, stimulating iNOS and c-fos via phosphorylation of MAPKs which may lead to lung tumour promotion [133]. Increased levels of both iNOS and neuronal NOS (nNOS) have been identified in human bronchial dysplasia suggesting an increase in cellular proliferation by oxidant-mediated mechanisms which the authors propose is associated with an early stage in carcinogenesis [134]. The potential importance of iNOS in carcinogenesis has been demonstrated in a study where iNOS was genetically ablated in mice resulting in a decrease in urethane-induced lung tumourigenesis [135]. VEGF expression was also reduced in the knockout mice, implying that increases in VEGF levels induced by the presence of NO can modulate angiogenesis, an important feature in tumour formation.

The link between inflammation and cancer has been well established and this link has been the subject of a number of review articles [136-139]. Inflammation is a physiological process that includes injury, repair and resolution in response to tissue damage/injury, infection and irritation [120, 140]. An inflammatory response involves the recruitment of inflammatory cells such as neutrophils, mast cells and monocytes by signalling cascades including growth factors, cytokines and chemokines. The inflammatory cells release growth factors, cytokines and other inflammatory mediators which can affect local epithelial, endothelial or mesenchymal cells [136]. This is followed by a large increase in oxygen uptake by these inflammatory cells (called a respiratory burst) and the subsequent release of free radicals [137, 140]. When inflammation occurs acutely it is therapeutic and self-limiting with anti-inflammatory cytokines controlling the process [136, 138, 140, 141]. However, when inflammation becomes chronic, it can provide an environment that promotes tumourigenesis via a number of mechanisms including increased cell proliferation, angiogenesis and DNA mutation [139].

Cigarette smoking has been reported to induce a state of chronic inflammation which can stimulate tumourigenesis [138, 141]. This is achieved by constant mediation of an oxidative stress response which stimulates pro-inflammatory cytokines, thus creating a pro-carcinogenic inflammatory response [120, 138, 140-142]. Cigarette smoke has been shown to induce pro-inflammatory cytokine release through NF-κB activation in macrophages [143]. Increased NF-κB activation and cytokine release was also observed in the lung in response to cigarette smoke, leading to an inflammatory environment that promotes carcinogenesis [144].

Cyclooxygenase 2 (COX-2) is a key inflammatory mediator that has been linked with carcinogenesis. COX-2 is an important inflammatory enzyme secreted by both inflammatory cells and non-inflammatory cells. It is overexpressed in many cancers including that of the lung [120, 145]. COX-2 activity can affect DNA mutation rates, cell proliferation, apoptosis, angiogenesis and metastasis [146]. It has been reported that cigarette smoke can induce the expression of COX-2 in lung fibroblasts [147], which may lead to an inflammatory environment and support the development of malignancy in the overlying epithelial cells. Recent *in vitro* studies demonstrating the overexpression of COX-2 in human lung fibroblasts in response to treatment with cigarette smoke extract have provided further support for this theory [148].

Further evidence of a link between cigarette smoke-induced oxidative stress and inflammation in carcinogenesis has come from studies on Clara cell 10-kDa protein (CC10). CC10 is ubiquitously produced in the lung by bronchiolar Clara cells. While its biological functions have not been fully established, it is thought to have a protective effect against inflammation and oxidative stress [149]. CC10 has been proposed to act as a tumour suppressor [150]. CC10 levels in plasma and bronchoalveolar lavage (BAL) fluid were shown to be higher in healthy non-smokers when compared to smokers and CC10 levels tend to increase upon smoking cessation [151]. This suggests that at least some of the damage induced by smoking is repaired once exposure to cigarette smoke ceases. A follow-up study by the same group formed part of a chemoprevention trial in smokers with a high risk for lung cancer [152]. The results of this study indicated that elevated levels of CC10 protein were significantly associated with regression of bronchial dysplasia [152]. Other oxidants have also been shown to decrease CC10 levels in the lung and this marker of oxidative stress has been proposed as an early indicator of carcinogenesis susceptibility [153]. Indeed, the use of inflammatory biomarkers in general has been suggested as providing both a prognostic tool and a basis of a therapeutic approach for cancer [139].

To summarise this section on cancer, the abundance of ROS under conditions of oxidative stress can drive carcinogenesis in a number of ways. These divergent mechanisms vary from the direct interaction of ROS with DNA and other biomolecules to their key roles as intracellular signalling molecules in pathways that are central to carcinogenesis. ROS can influence all stages of tumourigenesis from tumour initiation through to promotion and progression. The carcinogenic activity of cigarette smoke is due, at least in part, to the induction of oxidative stress and chronic inflammation by this complex chemical mixture.

CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Chronic obstructive pulmonary disease (COPD) is a major and growing cause of morbidity and mortality across the globe [154, 155]. It is defined as *'A preventable and treatable disease with some significant extrapulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterised by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and is associated with an abnormal inflammatory response of the lung to noxious particles or gases'* [156]. Although cigarette smoking has been recognised since the 1950s as the most important causative factor in the development of this disease [157], risk factors other than tobacco use have been identified [158]. According to the World Health Organisation approximately 80 million people worldwide have moderate to severe COPD and by 2020 it is estimated to become the fifth leading cause of death worldwide [156].

Pathologic changes characteristic of COPD are observed throughout the airways and pulmonary vasculature. In the central airways there is an increase in the number of inflammatory cells that populate the conducting airway epithelium [159]. Goblet cell numbers are increased and enlarged mucus-secreting glands, seen throughout the trachea and bronchi, lead to excessive mucus production. In the peripheral airways (airways that have a diameter less than 2 mm) chronic inflammation and repeated cycles of injury and repair result in abnormal remodelling [160]. Scarring, increased connective tissue deposition and mucus hypersecretion narrow the airways and lead to obstruction and airflow limitation [161]. In the airway regions, and in more severe COPD in the entire lung, dilatation and destruction of respiratory bronchioles (centrilobular emphysema) may be present. Increased thickening of the pulmonary vasculature intimal layer due to smooth muscle cell proliferation occurs during the early stages of COPD and is followed by an increase in inflammatory cell numbers [162, 163]. These changes lead to the characteristic features of COPD and include mucus hypersecretion, airflow limitation, pulmonary hyperinflation, gas exchange abnormalities and pulmonary hypertension. In advanced COPD peripheral airway obstruction, tissue destruction and pulmonary vasculature abnormalities with major cardiovascular complications reduce the lungs' capacity for effective oxygen exchange leading to a poor clinical outcome.

Risk factors for COPD include host factors and environmental exposures and the disease develops as a consequence of the interaction between the two. Oxidative stress, as a consequence of cigarette smoke exposure and endogenous up-regulation of metabolic processes, leads to the release of reactive oxygen and nitrogen species which is considered to contribute significantly to the pathogenesis of COPD [164]. In the following sections the role of oxidative stress, cigarette smoking and the development of COPD is explored.

OXIDATIVE STRESS AND COPD

During respiration the lung is exposed to a high-oxygen environment and inhaled toxicants such as cigarette smoke, occupational dusts and chemicals, and air pollution. This can lead to ele-

vated concentrations of ROS and RNS that interact with lung surface biomolecules. In addition, there are a number of endogenous sources of ROS that may further contribute to the increased oxidative burden in COPD. Activated inflammatory cells [165] attracted to the lung following tissue injury generate and release superoxide anions into the extracellular environment. These reactive species can undergo further reactions to form a variety of potent oxidants such as the hydroxyl radical ($HO^{\cdot}$) and hypochlorous acid (HOCl) [166]. Up-regulation of certain normal metabolic processes may further contribute to this oxidant burden. For example, the action of mitochondrial metabolism, molybdenum hydroxylase (xanthine sulphate and aldehyde oxidases) reactions and arachidonic acid metabolism can further increase oxidative stress [166]. These damaging reactive species are normally kept at bay by a multitude of enzymatic and non-enzymatic antioxidants, the types and concentrations of which have been extensively reviewed [167, 168]. However, it is the alterations to this finely balanced system that are important in the development of tissue injury, inflammation and subsequently lung disease.

Physiologically, COPD is characterised by a reduction in lung function as a consequence of airway narrowing. In a number of observational studies, low dietary antioxidant intake and low serum antioxidant concentrations have been associated with decreased lung function [169-172] and subsequently with increased COPD mortality [173]. Whether these observations reflect underlying biological mechanisms is unclear. However, they have been supported by studies that have addressed antioxidant gene-disease associations and more specifically the effect of genetic variants on the antioxidant-oxidant balance, both locally and systemically, in the pathogenesis of COPD. The GSTM1 (Glutathione S-Transferase) null genotype which results in a complete lack of enzyme activity [174] is associated with increased COPD risk [175-176]. The effect of the GSTP1 substitution mutation (Ile105val), which causes altered affinity for specific substrates [177] is also shown to be inversely associated with the development of the disease [178]. A rarer substitution in SOD3 which increases plasma superoxide dismutase (SOD) levels was also associated with a significantly decreased risk of developing COPD [179], whereas individuals that were homozygous for the E1/I1 polymorphisms in the same gene had reduced forced vital capacity (a measure of lung function) [180]. In support of these observations, animal studies in which transgenic mice overexpressing human SOD3 exhibited attenuation of lung damage and inflammation following exposure to hyperoxia [181]. These studies indicate that changes in the antioxidant status brought about either by dietary means or through genetic variation can lead to an alteration in the antioxidant-oxidant profile that can lead to alterations in lung function and an increased risk of developing COPD.

Markers of direct ROS-mediated effects such as carbonyl-modified proteins, DNA fragmentation and the formation of lipid hydroperoxides are all elevated in the BAL fluid, blood and lung tissue of subjects with COPD [182-186]. Oxidative stress renders proteins more susceptible to proteolytic degradation by modifying amino acid side chains, forming aromatic amino acid hydroxyl groups, quinines and protein aggregates [187]. During this process some residues are converted to carbonyls that can be measured in most biological matrices. These changes will often result in an alteration in the structure and function of the protein [188, 189]. This can lead to an alteration in antigenicity and immune responses, contraction of smooth muscle, impairment of receptor function, stimulation of airway secretion and cellular activation [7]. In a recent report by Torres-Ramos [183], significantly elevated concentrations of protein carbonyls were associated with COPD and these levels progressively increased with disease progression. Furthermore aldehydes generated from lipid peroxidation may react with the sulfhydryl and amino- moieties of plasma proteins [190]. This converts amino acids such as tyrosine into 3-nitrotyrosine and dityrosine, known indicators of free radical activity and protein damage [186]. Nitrotyrosine levels are increased in plasma and epithelial lining fluid of smokers and negatively correlate with FEV_1 (Forced Expiratory Volume in one second), a marker of lung function [191]. Proteinase inhibitors such as α1-proteinase inhibitor and secretory leukoproteinase inhibitor can be inactivated by ROS. Oxidative inactivation of a critical methionine residue in α1-proteinase inhibitor lead to a dramatic reduction in its inhibitory activity towards neutrophil elastase [192]. This process is thought to be critical in the pathogenesis of emphysema and COPD.

When generated close to cell membranes, ROS can induce lipid peroxidation with the subsequent accumulation of lipid peroxides and hydroperoxides. These then interact with various antioxidants or decompose after reacting with metal ions to form hydrocarbon gases and unsaturated aldehydes [166]. Aldehydes generated during this process may be involved in many of the pathophysiological events associated with increased oxidative stress [193] and are important in a number of inflammatory events within the lung. Lipid peroxidation products are found in elevated concentrations within the lungs of COPD patients [194] and negatively correlate with lung function [195]. Ethane, the volatile by-product of the peroxidation of fatty acids, is exhaled in larger quantities in COPD subjects [196] than controls. However, the origin of this by-product is uncertain as ethane can be generated in other organs of the body and excreted via the lungs [197], thus potentially implicating systemic oxidation as a contributory factor to the elevated levels observed. 4-hydroxy-2-nonenal (4-HNE) and the F_2-isoprostanes are specific lipid peroxidation products formed following oxidative stress. 4-HNE is a diffusible and highly reactive lipid which is a chemoattractant for neutrophils [198] and can also modify cell proliferation, cause T-cell apoptosis and activate various signalling pathways [199]. In fact, elevated levels of 4-HNE-modified proteins have been demonstrated in subjects with airway obstruction [200]. However, the exact role of this peroxidation by-product in the pathophysiology of COPD is yet to be clarified and further studies are required. The isoprostanes are a family of prostaglandin isomers which are required for full activity of the 5-lipoxygenase pathway. They are produced by the nonenzymatic lipid peroxidation of arachidonic acid and have a profound effect on a variety of cell functions, potentially by orchestrating cytokine expression, airway constriction, inflammation, epithelial permeability and mucus secretion [201]. Levels of 8-isoprostane measured in the urine negatively correlate with the severity of airway obstruction [202] and isoprostane $F_2\alpha$-III is elevated in COPD patients, with the highest levels being observed during disease exacerbations [203].

In chronic bronchitis, a feature often seen in subjects with COPD, there is increased mucus hypersecretion due to increased secretion from goblet cells and goblet cell hyperplasia. A large number of studies have demonstrated that ROS play a significant role in this process with increased expression of key mucin genes in the bronchial lumen and bronchiolar epithelium of subjects with COPD [204]. Oxidative stress, along with many other factors can cause goblet cell metaplasia and mucus hypersecretion [205] which may be initiated by epidermal growth factor receptor (EGFR) activation [206]. *In vitro* studies have shown that the administration of hydrogen peroxide to normal human nasal epithelial cells results in a significant over-expression of the mucin MUC5AC and internalization of EGFR. These studies demonstrate that oxidative stress brought about either by excessive exposure to exogenous or endogenous ROS and RNS and/or through the reduction in the antioxidant screen can lead to changes in lung function and increase COPD risk. In the next section the role of cigarette smoke in this process is discussed.

CIGARETTE SMOKE, OXIDATIVE STRESS AND COPD

Cigarette smoke is central to the development of COPD and accounts for 80-90% of all reported COPD cases. However, for unknown reasons only 10-15% of cigarette smokers will go on to

develop clinically significant disease [207, 208]. While the reasons for this are unclear, it is known that smokers who develop COPD exhibit an abnormal (enhanced) inflammatory response which fails to resolve after smoking cessation [209, 210]. The factors involved in this response are poorly understood and may involve genetic and epigenetic factors, altered immune regulation and abnormal repair mechanisms [7], primarily as a consequence of a reduced ability to combat oxidative stress [164, 166, 211].

As mentioned earlier, cigarette smoke is a rich source of reactive oxygen and nitrogen species in both the tar and gas phases of the smoke and is known to impact the levels of antioxidant within the lungs. Decreased total antioxidant capacity of blood following tobacco smoke exposure has been reported [212-214] and this has been confirmed in several *in vitro* and rodent studies [215]. The reduction in the antioxidant capacity of the blood is associated with disease severity and specifically with decreased levels of ascorbate, vitamin E, β-carotene, uric acid and selenium [216, 217]. Reduced plasma antioxidant levels appear to be associated with a family history of lung disease [169] but usually return to normal levels after smoking cessation. In sputum and BAL fluid glutathione levels are increased in patients with moderate and severe COPD, compared with healthy smokers [218, 219]. This indicates a potential up-regulation of antioxidants due to oxidative stress. This interplay between smoking history, age and antioxidant response is complex and further studies are required to fully elucidate the role of antioxidants in the development of cigarette smoke induced lung disease.

Exposure to cigarette smoke induces inflammation in the lungs of subjects with normal lung function [220]. Further, bronchial biopsies, sputum analysis and BAL fluid obtained from the airways of long-term smokers demonstrates the coexistence of airway and parenchymal inflammation in patients with symptomatic airflow limitation, thus suggesting a connection between cigarette smoking, inflammation and the subsequent development of COPD [221, 222]. The mechanisms by which cigarette smoke exposure elicits an inflammatory reaction are complex, but the oxidation of key biomolecules can lead to the attraction and activation of inflammatory cells such as neutrophils, macrophages and lymphocytes. These inflammatory cells may then further contribute to and cause progression of oxidative stress-induced tissue injury through the release of more ROS, cytokines and proteases. Neutrophils recovered from the blood of smokers and alveolar macrophages from BAL elaborated more superoxide than similar cells recovered from non-smokers [223, 224]. Additionally, superoxide generation from neutrophils correlated with bronchial hyperactivity in COPD patients [212]. This increase in oxidative stress in smokers can lead to oxidative changes in lipids, proteins and DNA as described above. In one study, exposure of human plasma proteins to the gas phase of cigarette smoke resulted in carbonylation [225]. In another, oxidised proteins were significantly elevated in the bronchoalveolar lavage fluid of asymptomatic smokers compared to non-smoking control subjects [182]. Similar changes in lipid peroxidation products have also been observed in cigarette smokers. Increased thiobarbituric acid-reacting substances are increased in the plasma and BAL of healthy smokers and patients with emphysema and chronic bronchitis. Interestingly these products correlated inversely with the degree of airway obstruction in COPD patients [186]. Non-invasive measurements of volatile lipid peroxidation products (ethane and pentane) are also elevated in cigarette smokers [197]. Many of these lipid peroxidation products are bioactive and may further contribute to lung injury, potentially through activation of a variety of pro-inflammatory genes. Recent data suggest that 4HNE can modulate biological processes in a variety of ways, possibly through the induction of heme oxygenase 1 (HO-1) and the subsequent activation of IL-8, a potent chemoattractant for neutrophils. However, this association between cigarette smoke and increased lipid oxidation products has not always proved consistent. A study by Morrow and colleagues [226] demonstrated elevated plasma levels of F_2-isoprostane in smokers, an observation not consistently seen in other studies. However, this may indicate the variability of response to oxidative injury between individuals and demonstrate that certain individuals are more resistant to oxidative stress, possibly through enhanced antioxidant defences.

To summarise, these and many other studies now implicate oxidative stress in the pathogenesis of COPD in patients who are cigarette smokers. However, host factors critical in disease susceptibility are only just being elucidated. Further research will undoubtedly clarify the factors involved in the host-environment interaction that leads to obstructive lung disease.

CONCLUDING REMARKS

In summary, cigarette smoke is a potent and well-defined source of oxidative stress, either due to the delivery of oxygen and nitrogen radicals in the smoke itself or as a stimulant of cellular free radical production. Many studies have been carried out both *in vitr*o and *in vivo* to examine cigarette smoke-induced oxidative stress, and the more frequent use of gene modification studies in the modern post-genomic era has yielded new mechanistic insight into how smoke induces oxidative stress and disease. Whatever the nature of the investigation, without doubt the overarching conclusion of the sum of these past efforts is a consensual opinion linking cigarette smoke and oxidative stress to smoking-related cardiovascular disease, COPD and cancer. The greatest challenge lies ahead and may lead to answers to the most prominent questions – can the oxidative stress burden delivered and induced by smoking be mitigated, and will this reduce the harmful effects of smoking and lead to a reduction in disease risk? Use of many of the techniques described throughout this review will potentially enable us to answer these questions, as well as providing greater knowledge on the actual components of the smoke which deliver the oxidative stress burden to smokers.

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