Micronutrient antioxidants and smoking

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Cigarette smoking is a major risk factor in such human diseases as cardiovascular disease (especially atherosclerosis), lung cancer (the leading world-wide cancer killer), and chronic obstructive pulmonary disease (COPD). An avalanche of studies has suggested that a diet rich in fruit and vegetables is associated with decreased risk for atherosclerosis and cancer. However, the dietary intake of fruits and vegetables, as well as antioxidant micronutrients, is decreased in smokers. This, along with evidence of increased utilization of ascorbic acid and α-tocopherol, possibly on the basis of increased oxidative stress, contributes to the low plasma antioxidant concentrations seen in many smokers. This review addresses selected mechanistic considerations of this relationship.

Cigarette smoke

Cigarette smoke (CS) is a mixture of over 4000 gas-phase, vapor-phase and particulate components. CS is known to contain many bioactive substances which undergo complex interactions with host extracellular and intracellular constituents, including lipid, protein and DNA, either before and/or after metabolism by the cytochrome P-450 and glutathione transferase and other xenobiotic metabolizing enzyme systems.

Although the chemical mechanisms underlying many of the effects of CS on biological systems are still poorly understood, CS toxicants are known to include redox-cycling quinones, polycyclic aromatic hydrocarbons (PAHs), aromatic and heterocyclic N-nitrosamines, and stable and unstable reactive oxygen and nitrogen species. There is increasing evidence that oxidative and nitrosative stress is an important event in the incidence and/or severity of several of the CS-related diseases, including both atherosclerosis and cancer. It is thus not surprising to find a plethora of studies focusing on interactions between CS-related direct oxidative and nitrosative stresses, augmented by host activation of inflammatory-immune systems, and the role that dietary antioxidants...
might play in ameliorating (or in some cases, paradoxically, potentiating) CS-related diseases. Figure 1 represents a simplistic scheme depicting this interaction.

Cigarette smoke and inflammatory-immune system activation

CS-induced respiratory tract injury involves the activation of inflammatory-immune cells initiated by the primary and secondary CS related processes. These are important defenders of the lung against environmental pathogens, but can injure the lung, in part as a result of their generation of reactive oxygen and nitrogen species. This CS-related activation of systemic inflammatory-immune processes is likely to be related to atherogenesis, and possibly to carcinogenesis.

Of relevance to this general concept are the findings that smokers have increased numbers of circulating phagocytes, and that these phagocytes have ‘primed’ oxidant generating systems. Numerous models have shown that CS exposure increases leukocyte, platelet and monocyte adhesion to endothelial cells and platelet aggregation, events which have been linked to atherosclerosis. Importantly, this increased adhesion is
Micronutrient antioxidants and smoking decreased by micronutrient and enzymatic antioxidants\(^ {17-19}\), and by inhibitors of platelet activation factor pathways which are related to lipid peroxidation\(^ {20}\). In several experimental models, antioxidants were found to suppress phagocyte-related oxidant production, but the clinical implications of these findings remain uncertain\(^ {21}\).

Endothelial cells are dynamic participants and/or targets in inflammatory-immune processes\(^ {22,23}\) including atherosclerosis\(^ {24-27}\). Endothelial cell-dependent vasodilatation is impaired in chronic smokers, and this is in part reversible by antioxidant micronutrients\(^ {27}\). There is a large body of evidence that implicates nitric oxide (NO) as a modulator of both adhesive interactions between cells that participate in inflammatory responses and endothelial cell-dependant vasodilatation\(^ {24-26}\). Inhibition of NO synthesis increases vessel wall inflammation\(^ {28}\), whereas stimulation of these pathways inhibits endothelial dysfunction in smokers\(^ {29}\). Perturbations of NO biosynthesis and reactivity, either related to the high levels of NO in CS or by other CS components, play a yet to be fully clarified role in endothelial cell dysfunction seen in chronic smokers.

Numerous epidemiological studies have demonstrated an association between systemic markers of activated inflammatory-immune processes, such as elevation of C-reactive protein and intercellular adhesion molecules and neutrophil counts and overall mortality from atherosclerosis\(^ {30}\). Inflammatory processes themselves are being increasingly linked to many stages of atherosclerosis\(^ {27}\). For example, inflammatory reactions in active atherosclerotic plaques (including even possible infections and/or immune activations) have implications for plaque rupture and thrombosis\(^ {27,31}\), and as smokers have activated inflammatory-immune systems, this may be one mechanism whereby continued smoking promotes plaque instability. However, hemorheologic factors are also believed to play a role in thrombotic phases of this important clinical event\(^ {32}\). These active plaques can be detected by thermal detection techniques\(^ {33}\). Inflammation and its accompanying prooxidant processes have also been implicated in many stages of carcinogenesis\(^ {34}\), including lung\(^ {35}\) and pancreatic cancers\(^ {36}\).

Issues remaining to be clarified include what CS gas and particulate phase interactions with lung tissues occur, what products are produced, and which of these products can be transferred into the systemic circulation and affect vascular and non-lung tissues. How these transferred products result in the various CS-related pathobiologies, including pro-inflammatory system activations, atherosclerosis, carcinogenesis, and COPD remain to be demonstrated.

**Cigarette smoke and atheriogenoesis**

Atherosclerosis begins with intimal depositions of low-density lipoprotein (LDL) which are taken up by monocyte/macrophages to form the fatty
streaks found in the arterial walls. As some of these lesions continue to accumulate intracellular and extracellular lipid, a fibromuscular cap forms above the intima, and raised lesions develop. The raised lesions can progress to advanced complicated lesions including plaques. Vulnerable active plaques containing active inflammatory cells can undergo destabilization and rupture, triggering inappropriate platelet activation and thrombus formation, leading to ischemic clinical events.

Epidemiological, morphological, and functional studies have shown that CS is an independent risk factor for atherosclerotic disease. The precise multifactorial mechanism(s) by which CS produces these effects remain unclear. However, there are many stages where CS could potentiate atherosclerotic processes, and of antioxidant micronutrients could target one or more of these processes.

Oxidative modifications of subendothelial lipoproteins dominate current concepts regarding atherogenesis. A plethora of studies have measured the oxidative susceptibilities of circulating LDL using traditional indices of lipid peroxidation in vivo, many (but not all) of which have shown that smokers have increased susceptibility and that both non-smokers and smokers have decreased susceptibilities upon antioxidant supplementation. However, these studies do not mimic the conditions in the intima of large arteries where lipid oxidation mechanisms are likely to be more complex, and thus, studies of circulating LDL oxidizability are probably of limited value until they are shown to be related to disease outcomes. The actual factors that initiate and propagate peroxidation in the intima, and the effects of antioxidant interventions in that milieu, are not fully understood, nor are the effects of the peroxidation products on atherotic plaque pathogenesis fully elucidated. These products might influence a wide variety of biological processes, including the inflammatory and fibroproliferative responses which characterize atherogenesis.

The use of specific novel products of in vivo lipid peroxidation, such as the F2-isoprostanes, prostaglandin isomers that result from oxidative modification of arachidonic acid through free radical reactions, have provided evidence that lipid peroxidation is increased in smokers and that this can be ameliorated by antioxidant supplements. This work is strengthened by studies done under controlled feeding conditions showing that expired breath ethane, another measure of lipid peroxidation, is higher in smokers compared to non-smokers, although these data are confounded by the fact that ethane is found in CS. Although this approach has yet to be linked with the presence and/or severity of atherosclerosis in humans, it is relevant that such strategies are being used in animal models of atherogenesis, strengthening the validity of these approaches. Eventual linkage with non-invasive clinical staging of atherosclerosis, using such modalities as blood tests specific for atherosclerosis, high resolution vascular ultrasonography, computed tomography,
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magnetic resonance imaging angiography, and radionucleotide managing\(^*\), should be one goal of these approaches.

A recent study has meticulously compared the prevalence and extent of atherosclerosis in both smoking and non-smoking trauma victims 15–34 years of age\(^{47}\). This study, not surprisingly\(^{48}\), showed that smokers had twice as many advanced lesions (e.g. raised lesions with fibromuscular proliferation and ultimate plaque formation) than did non-smokers, but somewhat surprisingly showed smokers to have fewer early lesions (e.g. fatty streaks) than non-smokers. The overall prevalence of lesions was similar, indicating that, in smokers, early and intermediate lesions progress more rapidly into advanced lesions. This study further suggests that CS has little influence on the early development of fatty streaks, but accelerates the progress from fatty streaks to advanced lesions. Hence, if antioxidants are effective in ameliorating accelerated atherogenesis seen in smokers, oxidative processes are probably influencing this progression, although it is possible that selected antioxidants are affecting more than antioxidant pathways (e.g., vitamin E may affect protein kinase C activity)\(^{21}\). It should also be recognized that it is the vulnerable atherosclerosis plaque instability and rupture, a complex and dynamic process in itself\(^{27,31}\), that often causes the acute ischemic event, and that there is some evidence that antioxidants influence both plaque instability/rupture and subsequent clot formation\(^{49}\).

Importantly, smokers also have increased platelet reactivity\(^{50}\), often the terminal event in the atherosclerotic cascade, and this reactivity may be influenced by antioxidants.

Functional studies of blood vessel reactivity in smokers have supported the view that oxidants play a role in CS-related atherogenic processes. Endothelial dysfunction, as manifested by impaired vasodilatation and seen in the presence of most cardiovascular risk processes, including smoking, is believed to contribute to the development of atherosclerotic diseases\(^{10-12,27}\). Oxidative stresses, probably involving NO and superoxide anion generations and cyclooxygenase and lipoxygenase pathways, represent interacting systems that in large part account for this dysfunction\(^{51,52}\).

Cigarette smoke and cancer

CS contains numerous proven and suspected mutagens of which toxins such as nitrosamines and polycyclic aromatic hydrocarbons (PAHs) have received the greatest attention\(^{9,53}\). As with atherosclerosis, CS-related cancers are the result of complex multi-stage processes of which hereditary susceptibility, environmental co-exposures (e.g. radon, asbestosis) and diet are recognized factors. In order to understand how CS causes lung cancer (and other CS-related cancers), and how antioxidant micronutrients could influence this process, it is necessary to understand the
sequence of molecular events that lead from CS inhalation and tissue responses to the clinical presentation of tumors decades later. The sequence of events is confounded by the complexities of CS constituents (and their tissue interactions) and the genetic variabilities of the host responses, including those of inflammatory-immune systems, xenobiotic biotransforming systems, and the myriad of host DNA repair systems.

CS carcinogenesis involves interactions between mutagens (e.g. PAHs, nitrosamines, redox cycling amines), their activations by phase I cytochrome P-450 drug metabolizing systems and detoxifications by phase II enzymes (e.g. glutathione transferases and epoxide hydrolases), and the host genome repair systems. Superimposed on these interactions are genomic modulations affected by CS-related activations of inflammatory-immune systems and cellular signal-generating systems which modulate host gene expressions related to various stages of tumor initiation and progression. Susceptibility polymorphisms in the phase I\textsuperscript{55} and phase II\textsuperscript{56,57} biotransforming systems have been assessed with regard to their role in modulating both DNA adduct levels and CS-related cancer incidences. Although these and several other studies have focused on considerations of CS-derived adducts\textsuperscript{58-60}, surrogate markers which are presumably related to early stages of CS-related carcinogenesis (although this link needs to be more firmly established), few studies have addressed how dietary antioxidants might influence adduct formation and health-related outcomes. Surprisingly, recent data have shown that CS-related DNA damage may be involved in coronary artery disease\textsuperscript{61}.

Other studies have focused on 8-OH-deoxyguanosine levels as an indicator of oxidatively modified DNA and have shown 8-OH-deoxyguanosine levels to be increased in tissue, blood and in urine of smokers\textsuperscript{35,36,62-65}. In many\textsuperscript{63,65}, but not all\textsuperscript{64}, of these studies, antioxidants decreased these levels, as did smoking cessation\textsuperscript{66}. However, these studies are yet to be rigorously linked with cancer epidemiology studies focused on pathogenesis and incidence of disease. Importantly, antioxidant nutrients are likely to interact at multiple levels of complex interacting carcinogenic processes, and many yield both beneficial and adverse effects depending on the specific endpoint being scored\textsuperscript{67}.

As is the case for atherosclerosis, there is considerable evidence for an association between diets rich in fruits and vegetables and a decreased overall cancer risk\textsuperscript{3,13,68}. It is often assumed that antioxidant micronutrients in fruits and vegetables contribute to this decrease. However, mechanistic details of this interaction remain sketchy. For example, several potent CS-related carcinogens are bioactively modified by metabolic activation/detoxification pathways which themselves might be modified by antioxidants.

The complexities of the relationships between CS-cancer and antioxidant micronutrients is, of course, best illustrated by the adverse and
contradicting results of several recent intervention trials which have shown that assumed antioxidant micronutrients supplements (e.g. β-carotene) increased the risk of acquiring lung cancer, at least if administered to heavy smokers during the more advanced preclinical stages of the carcinogenic process\textsuperscript{69,70}. Moreover, in an animal model β-carotene itself was shown to increase precancerous lesions in the lungs of both ‘smoking’ and non-smoking ferrets\textsuperscript{71}, possibly related to actions of its oxidative products\textsuperscript{72}. These studies have much invigorated basic research on biochemical activities of antioxidants.

There is thus far little evidence that factors involved in genetic instability and mutagenesis, often assumed to be prerequisites for initiation, are the same factors required for cancer progression and eventual metastasis. At the other end of the spectrum, over half of lung cancers are now appearing in former smokers and there is little information concerning the role of antioxidant micronutrients in the chemosuppression of these tumors. Finally, although CS constituents and host inflammatory-immune and biotransforming system modulators might collectively be well suited to influence all phases of tumorigenesis, it is unlikely that antioxidant micronutrients would be capable of equally influencing all stages of this complex process.

Cigarette smoke and chronic obstructive pulmonary disease

CS is the most important factor in the aetiology of COPD. Although imbalances between proteases and antiproteases are thought to be the primary causes in its pathogenesis\textsuperscript{73}, evidence for oxidative stress continues to emerge\textsuperscript{74,75}. Both cigarette smokers\textsuperscript{41-43} and smoking and non-smoking patients with COPD\textsuperscript{76} have evidence of increased lipid peroxidation. In COPD, exacerbation of the disease increased F\textsubscript{2}-isoprostanes levels\textsuperscript{76}, suggesting a role for inflammatory oxidant pathways. Further evidence suggest that CS causes oxidative damages in smokers’ lungs includes the presence of genetic damage in both nuclear and mitochondrial genomes of smokers’ lung macrophages\textsuperscript{77}. Others have shown dose-related correlative increases in lipid-peroxide content, mitochondrial DNA lesions and decreases in pulmonary function in lungs of smokers\textsuperscript{78}. In fact, CS itself causes lipid peroxidation in airway epithelium\textsuperscript{79}.

Consideration that oxidants might be involved in pathogenesis of COPD are buttressed by findings that levels of fresh fruit consumption are positively associated with increased pulmonary function\textsuperscript{80}, and that an association exists between low antioxidant intakes and lower plasma antioxidant levels and an increased incidence of adult symptoms of wheezing\textsuperscript{81}. Further, animal studies suggest that antioxidant administration might reduce CS-related lung damage\textsuperscript{82}. 
Marijuana smoke

Few studies have addressed the effects of marijuana on the incidences of the CS-related diseases, and such studies are often confounded by the fact that most marijuana smokers also smoke tobacco. Evidence is accumulating that marijuana smoke exposure is capable of inducing most of the CS-associated diseases, although almost no studies of the possible modifying effects of antioxidant micronutrients exist.

Secondhand smoke

Evidence continues to accumulate that environmental tobacco smoke (ETS) is weakly but significantly linked to atherosclerosis, cancer and chronic airway obstruction. Although exposure to ETS may induce atherogenic changes in LDL, few rigorous studies that have been designed to evaluate the effects of micronutrient antioxidants on these health effects. Importantly, there is accumulating evidence that employees exposed to secondhand smoke have increased levels of 8-OH deoxyguanosine in their blood that and smokers pass on CS constituents including potential carcinogens to the fetus.

Smokeless tobacco

Numerous studies have demonstrated that habitual use of smokeless tobacco induces mucosal and periodontal lesions at the sites of tobacco placement, presumably in response to components of tobacco. The sequence of inflammatory immune system activation (including mediator and cytokine responses) that eventually results in the characteristic lesions showing epithelial hyperplasia and lymphatic infiltration, and eventual cancer in some subjects have been described. As yet, the potential role of antioxidants in ameliorating effects of the antioxidant micronutrients on this process has not been studied.

Antioxidant micronutrient supplementation: remaining enigmas

The health benefits of antioxidant supplementation, for both normal subjects and for smokers, are not at all clearly established. Perhaps the best data come from studies of the role of vitamin E in patients who
already have heart disease. Here the data from epidemiology, intervention, and basic science studies all begin to fit together. Nonetheless, there is only one intervention trial that demonstrates that vitamin E supplementation decreases the risk of second non-fatal heart attacks; there was no effect on fatal heart attacks\textsuperscript{91}. Thus, the overall benefit of this well studied case is not at all clear, and the effect of other antioxidants or combinations of antioxidants have not been well studied. The results of studies showing increased incidences of CS-related lung cancers in relatively short-term studies of β-carotene supplementation signals a warning that large scale prevention trials can yield surprises\textsuperscript{91-93}. From a nutritional point of view, plasma ascorbic acid level is low in many smokers, and an increased consumption of vitamin C-rich foods is well advised.

Obviously, the basic molecular mechanisms by which CS increases the incidence and/or severity of atherosclerosis and the incidences of cancer need to be delineated in order to clarify the sites where antioxidants can ameliorate effects, and the concentrations of antioxidants that are effective \textit{in vivo} need to be specified. Clinical trials examining for beneficial effect of antioxidants in the CS-related diseases will have to take into account the stage of the disease being studied. The role of platelets is a case in point where certain F\textsubscript{2}-isoprostanes not only are markers of lipid peroxidation, but also are potent platelet aggregating agents\textsuperscript{94}. Here, administration of vitamin E potentially could decrease isoprostane levels, decrease expression of adhesion molecules in both platelets and endothelial cells and, thereby, decrease thrombus formation and potentially ameliorate the consequences of end stage atherosclerosis (\textit{e.g.} stroke and heart attack).

The explosion of information emerging on molecular biology and lung disease, genetic basis of atherogenesis, and carcinogenesis, utilizing such techniques as transgenic animal models, and newer techniques to quantitate atherogenesis and carcinogenesis at stages of their development are producing a wealth of new data which will predictably yield important new nuances relevant to CS-related athrogens and carcinogens and the CS-related human diseases. This should be especially true in defining many of the molecular and cellular changes that occur during early preclinical stages of the CS-related diseases and how antioxidants might influence these stages. Such studies should have to take into account such issues as genes within an individual which might influence the etiological course of the specific CS-related disease and the validation of the relevance of CS-related biomarkers to health outcomes. It is through concerted efforts in such fields as molecular epidemiology and nutrition that advances in new biology will rationally be translated into solid recommendations concerning the role of micronutrient antioxidants and the health of the smoker. The best
recommendation continues to be to remove the causative factor, CS itself.

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