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Topical Review

The free radical basis of air pollution: focus on ozone

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Introduction

Until the middle of the present century, urban populations in the United Kingdom suffered major health problems from air pollution, due principally to the burning of coal for domestic and industrial purposes. Following the disastrous London smog in 1952, from which an estimated 4000 people died, the Government introduced the Clean Air Act of 1956 to reduce the emission of recognized pollutants. As a consequence, deaths directly attributable to air pollution have largely disappeared. Control of the 'traditional' pollutants has, however, coincided with the appearance of a new pollutant of the atmosphere, ozone. The magnitude of this emerging problem has, until recently, been unappreciated and most probably underestimated. It is now recognized that safety guidelines for atmospheric ozone concentrations are commonly exceeded during summer months over much of Europe, an observation which has aroused considerable concern over possible effects on health (1).

A number of excellent reviews have been published regarding the impact of ozone on Public Health and, in particular, the respiratory system (2,3). The purpose of this review is not to readdress these areas but rather to examine the evidence for the mechanism of ozone toxicity, and in particular to focus on its ability to initiate free radical reactions, and to discuss the extracellular antioxidant defences in lung epithelial lining fluid which may modulate its toxicity.

Ozone and the Respiratory System: The Free Radical Basis of Ozone Toxicity

As a gaseous pollutant, the only significant route for exposure to ozone is by inhalation. Its primary target tissue is therefore the lung and it is reasonable to assume that the majority of its toxic effects are limited to this organ (4), although some extrapulmonary effects have been reported (5,6). Alterations in pulmonary function, along with the cellular and biochemical endpoints characteristic of exposure to ozone, have been studied in great detail both in human subjects and in animal models. However, despite this wealth of information, little is known about the mechanisms underlying ozone-induced tissue injury.

Ozone, though not a radical species itself ($0-0=0$), is thought to mediate many of its toxic effects through free radical reactions (7); either directly by the oxidation of biomolecules to give classical radical species such as ·OH, or by driving radical-dependent production of cytotoxic, non-radical species such as aldehydes and ozonides. Production of free radicals, in both *in vitro* and *in vivo* models, has been clearly demonstrated upon ozone challenge (8). Furthermore, the formation of products characteristic of free radical damage has been shown to be prevented or delayed upon the addition of vitamin E, in a wide range of experimental systems employing ozone (9,10). Ozone will potentially react with all hydrocarbon molecules (11), although its reactivity towards specific groups varies over several orders of magnitude. This ubiquitous, yet hierarchical target specificity, allied to the limited aqueous solubility and diffusability of ozone, profoundly influences our understanding of the interaction of ozone within the lung.

As a result of its high reactivity, ozone is not believed to reach, and hence react directly with, the lung epithelium (11). Instead, ozone is thought to be consumed by reaction with components of the pulmonary epithelial lining fluid (ELF), a thin aqueous layer secreted by, and overlaying, the pulmonary epithelium (Fig. 1). Therefore, cellular damage is not

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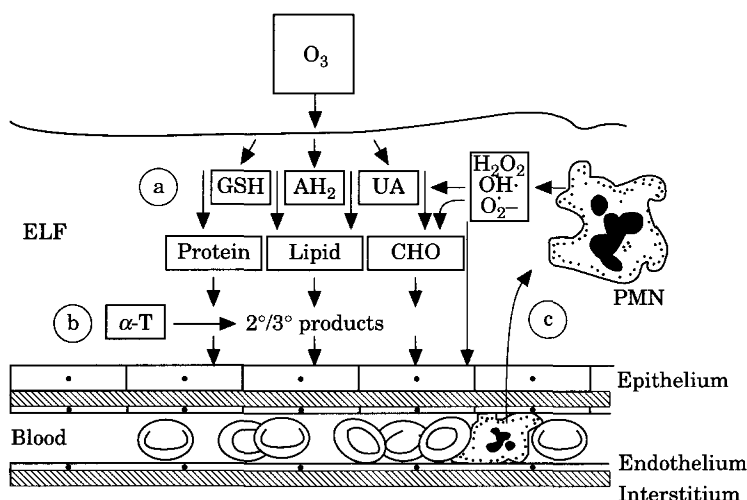

Fig. 1 Possible mechanisms of ozone-induced injury of the lung. Schematic representation of the interactions between ozone (O_3) and components of the epithelial lining fluid (ELF). (a) O_3 will react with all hydrocarbons but the intrinsic reaction rates between it and the water soluble antioxidants, reduced glutathione (GSH), ascorbate (AH_2) and urate (UA) are particularly fast. This allows these antioxidants to act as a first tier of defence against O_3-induced oxidant damage. They act as sacrificial substrates scavenging O_3 and thereby preventing oxidation of macromolecules such as lipid, protein and carbohydrate (CHO). When these antioxidant defences are overwhelmed by the oxidative burden, O_3 then reacts with these macromolecules and this results in the generation of secondary and tertiary products, many of which are cytotoxic. (b) Vitamin E (α-tocopherol; α-T) provides a second tier of defence by scavenging peroxyl radicals and preventing the propagation of free radical reactions. (c) O_3 exposure results in airway inflammation and activated polymorphonucleocytes (PMNs) marginate into the ELF and provide an additional source of reactive oxygen species, which further impinge upon the oxidative burden experienced in this micro-environment.

perceived as being a consequence of ozone *per se*, but rather as being mediated through a cascade of secondary, free radical derived, ozonation products. Irrespective of the exact source of these oxygen radicals *in vivo*, cellular damage arising from the imposition of an oxidative burden requires that the pulmonary antioxidant defences, both extracellular within the ELF and intracellular within the respiratory epithelium, are overwhelmed by the challenge.

Pulmonary Defences Against Ozone

Recently it has become clear that ELF forms the first line of defence against inhaled toxins such as ozone (12). Studies employing ^{18}O-labelled ozone have conclusively demonstrated that proportionally more ozone is taken up within the extracellular environment compared with either the bronchoalveolar (BAL) cell fraction or lung tissue of ozone-exposed rats (13). The reaction of ozone within the ELF can also be inferred from the detection of ozone-specific oxidation products in BAL fluid following *in vivo* exposure.

Given that the majority of ozone reacts within the ELF compartment, the antioxidant composition of this fluid is critically important in determining an individual's sensitivity to ozone. The antioxidant components of ELF include sulphydryl compounds, such as reduced glutathione, ascorbic acid, uric acid and vitamin E. Reported concentrations of these antioxidants in ELF are shown in Fig. 2.

THE GLUTATHIONE SYSTEM

Lung ELF contains high concentrations of reduced glutathione (L-γ-glutamyl-L-cysteinylglycine; GSH). In human subjects, GSH concentrations of 400 μmol l^{-1} have been reported (14), a value which is approximately 100 times that found in plasma. The source of this enormous GSH pool is not yet known but cellular secretion is probably involved, coupled with poor reabsorption mechanisms. A number of cell types, including mononuclear phagocytes, lymphocytes and fibroblasts, export GSH (15,16). Furthermore, the cell-surface enzyme responsible for the uptake of GSH from the extracellular space, γ-glutamyl transpeptidase, is present in lung at far lower concentrations than in other organs (17). In addition, lung epithelial lining fluid is replenished relatively slowly, such that any GSH exported into this environment is likely to remain there for prolonged periods, which may also contribute to its build up.

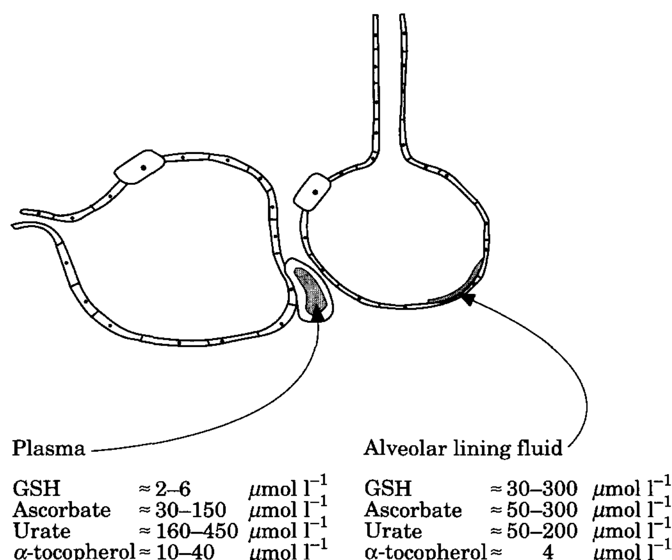

Fig. 2 Typical concentration of antioxidants in plasma and lung epithelial lining fluid. These values are based upon concentrations measured in bronchoalveolar lavage (BAL) fluid obtained from healthy subjects. Two important provisos should be made with respect to the concentration quoted. First, values quoted in the literature vary considerably, reflecting both subtle differences in subpopulations studied, the lavage technique employed and the problem of variable return of BAL fluid. Second, the BAL fluid obtained may contain epithelial lining fluid from both the peripheral conducting airways as well as alveoli. This second point is very important as the antioxidant and protein concentration of airway lining fluids can vary considerable throughout the length of the respiratory tree.

Ozone reacts with low molecular weight sulphydryl compounds, including GSH, as well as proteins with exposed sulphydryl groups (P-SH), oxidizing these to GSSG and PSSG, respectively. Using a combination of stop-flow and competitive oxidation measurements, Pryor *et al.* (18) have demonstrated that at physiological pH, the reactions of ozone with cysteine and GSH proceed with extremely large reaction rates; $1{\cdot}6{*}10^9$ and $7{*}10^8$, respectively. Furthermore, they have demonstrated that cysteine residues inhibit oxidation of tryptophan residues in ozone-exposed lysozyme (19). These findings support the conclusion that sulphydryl compounds are an important target for ozone, and that given their high concentration in ELF, they represent an important first line of defence against oxidative air pollutants. These observations are however at odds with the findings of other groups. Cross *et al.* (20) reported that when plasma was exposed to a high concentration of ozone (16 ppm), ascorbic acid and uric acid were preferentially depleted compared with protein sulphydryls, a result quite the converse to that reported by Giamalva *et al.* (21). Recently, we have obtained a similar hierarchy of antioxidant depletion to that reported by Cross (uric acid/ascorbic acid>GSH), for both human BAL fluid and pure biochemical solutions exposed to ozone (22,23). Further support for such a hierarchy have come from studies by Kanosfky and Sima (24–26).

When GSH alone is exposed to ozone, a stoichiometric 2:1 conversion of GSH to GSSG occurs in an ozone concentration and time-dependent manner (22). However, in more biologically relevant models, this simple stoichiometry does not hold. DeLucia *et al.* (27) have shown that rats exposed to 0·4 ppm ozone progressively lose lung GSH without a concomitant rise in GSSG. Instead, it is likely that a mixed disulphide between protein-SH and GSH occurs. O'Neill *et al.* (28) reported that the rate of -SH oxidation is critically affected by pH, being much faster at physiological pH than under acidic conditions. As ozone pollution is often associated with acidic aerosols, they argue that given the small volume of ELF, a drop in ELF pH could occur and this could seriously diminish the ability of GSH to scavenge ozone.

Though the concentration of GSH is very high in ELF, it is not yet clear whether the enzymatic components of the glutathione redox cycle operate in this micro-environment. Intracellular GSH is readily resorted after oxidation through the action of glutathione reductase with the concomitant use of NADPH. In the absence of tissue damage, and the release of these intracellular enzymes into ELF, these

enzymes do not appear to be present. Glutathione may therefore function in a sacrificial mode in ELF, reacting directly with ozone to give thiyl radicals (GS·) which can subsequently be converted to oxidized glutathione (GSSG) or GSS-P, through a radical transfer process, the details of which are not fully understood (29,30).

Epithelial lining fluid GSH concentration has also been shown to increase in rats in response to exposure to ozone (31). This response undoubtedly forms part of the mechanism for the mitigation of the toxic free radical mediated action of ozone. Whether this increase represents the consequence of increased *de novo* synthesis by particular cell types, or an absolute increase in the number of GSH synthesizing cells remains unknown. In 1992, Boehme *et al.* (32) examined the relationship between ELF GSH concentration and that of the cells resident in the lower airways in a rat model exposed to 0·8 ppm ozone for up to 7 days. They found that after an initial increase in the concentrations of glutathione in the resident cell populations, predominantly alveolar macrophages, the ELF GSH concentration was maximal at Day 7. This finding would support the concept that antioxidant capacity is transferred to ELF, where it is of greatest benefit to the lung. However, whether this process is active and directed or simply a consequence of oxidative cell lysis of the resident macrophages remains unresolved.

ASCORBIC ACID

Vitamin C or L-ascorbate is an important water-soluble antioxidant, capable of scavenging a variety of free radicals and oxidants *in vitro* (33–35). In humans, ELF contains varying amounts of ascorbate (36). As this water-soluble antioxidant is probably freely diffusable, dietary intake of vitamin C may play an important role in dictating ELF ascorbate concentration. *Ex vivo* studies with human lavage fluid have clearly shown that ascorbate is consumed in a ozone concentration and time-dependent manner (36). In these studies, and parallel ones conducted using pure biochemical solutions, the rate of consumption of ascorbate was significantly greater than that of GSH suggesting that vitamin C is an important component of the protective antioxidant screen present in ELF. When plasma was exposed to ozone *ex vivo*, ascorbate was also shown to be consumed in preference to plasma-SH groups (20). Interestingly, O'Neill found that the ability of ascorbate to scavenge ozone was not pH-sensitive between the range 5·0–7·4, again supporting its superiority over GSH in acting as an antioxidant buffer, even in co-pollutant challenges (28). This observation contrasts that made by Kanofsky *et al.* (26) in which the ozone–ascorbate rate constant, assessed via the fractional uptake of ozone, varied by two orders of magnitude between pH 2·0 and 7·0.

Intracellularly, ascorbate interacts in a number of important ways with other antioxidants. It is not presently known to what extent these reactions occur in lung lining fluid. During its scavenging activity, ascorbate undergoes an electron oxidation with the formation of the semi-dehydroascorbate radical (37). This radical is subsequently reduced by GSH to dehydroascorbate, but the reaction gives rise to a thiol radical (GS·), and presumably GSSG. As dehydroascorbate can subsequently be metabolized to the cytotoxic derivative oxalate, most cells contain the enzyme dehydroascorbic reductase which catalyses the regeneration of ascorbate from dehydroascorbate at the expense of the formation of GSSG. In addition to its direct scavenging action, ascorbate is also thought to act indirectly to prevent lipid peroxidation through its reaction with membrane tocopherol. It has been demonstrated *in vitro* that ascorbate is able to decrease the concentration of the tocopherol radical (38) with the regeneration of its non-radical form, thereby restoring its scavenging activity (39,40). It has therefore been proposed that the interaction of membrane-bound tocopherol radicals with ascorbate not only restores the antioxidant defences of the membrane, but also transfers the oxidative challenge to the aqueous phase of the cell where the resultant ascorbate radical can be eliminated by the processes outlined above. This synergistic action, though clearly demonstrated *in vitro*, has not yet been reported *in vivo*.

Importantly, ascorbate may also act as a pro-oxidant under certain conditions, permitting the reduction of Fe^{3+} to Fe^{2+}, thus potentiating the formation of hydroxyl radicals via Fenton chemistry (41). Whether ascorbate acts as an antioxidant or pro-oxidant is critically dependent upon the availability of free transition metal ions. In extracellular environments where metal ions are either efficiently sequestered into proteins, as in the plasma, or are present at only trace concentrations, as in ELF (under normal circumstances), ascorbate is considered to be a very important antioxidant (42,43).

Studies in guinea pigs, which like man cannot synthesize ascorbic acid, have demonstrated that ozone-induced lung damage is potentiated in ascorbate-depleted animals and that protection can be afforded by vitamin C supplementation in a dose-dependent manner (44). In animals capable of synthesizing ascorbate, exposure to ozone has been shown to increase the concentration of ascorbate in

the lung, though the underlying mechanism is unknown.

URIC ACID

Uric acid is an oxidized purine base which has been shown to have considerable free radical scavenging activities *in vitro* (47). It has attracted considerable attention since it was found to confer resistance to oxidative damage in the upper airways. This is partly as a result of its high concentration in this region (45) and partly because it may be utilized preferentially to other antioxidants. For example, studies in plasma have shown that it is used preferentially when exposed to high concentrations of ozone (46). Uric acid interacts directly with ozone and is consumed in a suicidal fashion. Studies with uric acid alone (23), in lung lining fluid (22) and plasma (46) have all demonstrated that it is consumed in a dose and time responsive manner by ozone and the rate of consumption is pH independent between 5·0 and 7·4 (28).

Uric acid can directly scavenge hydroxyl radicals (48), oxohaem oxidants formed by the reactions between haemoglobin and peroxyl radicals (49), peroxyl radical themselves (40) and singlet oxygen (49). It can also scavenge myloperoxidase-derived hydrochlorous acid (50) and prevent free radical damage to DNA (51). It appears to act in these reactions in a sacrificial mode – i.e. becoming irreversibly damaged itself through the interaction.

VITAMIN E

Whereas the water-soluble antioxidants, ascorbic acid, uric acid and to a lesser extent GSH, provide protection by acting as sacrificial targets for ozone, the rate constant for the reaction of the fat soluble antioxidant, vitamin E, with ozone is not sufficiently great compared with polyunsaturated fatty acids (PUFAs), for it to function in this fashion. Furthermore, as ELF contains substantially more PUFAs than vitamin E, fatty acids rather than vitamin E, probably react more frequently with ozone. Vitamin E is therefore unlikely to react directly with ozone but rather it scavenges peroxyl radicals derived from ozone/PUFA interactions. The interaction between membrane-bound vitamin E and ozone-derived radicals has been studied extensively due to the perceived importance of lipid peroxidation in ozone-induced lipid injury (see later). The addition of vitamin E to ozone-exposed PUFAs systems abolishes the electron spin resonance radical profiles indicating its radical scavenging activity (9).

Ozone exposure studies on vitamin E deficient animals have shown that: (i) at otherwise lethal concentrations, survival is increased by vitamin E supplementation; (ii) supplementation results in the depression of glutathione redox and pentose phosphate enzyme activities otherwise elevated as a result of ozone exposure (52,53); (iii) Heinz body formation is decreased by supplementation (54); (iv) GSH concentrations are lower in deficient animals compared with animals given supplements; and (v) vitamin E supplementation appears to protect against cell damage (55). In addition, the concentration of lung vitamin E has been reported to be elevated in short-term exposures to ozone (56).

Despite the evidence supporting a protective role of vitamin E against oxidative stress, it has not yet been demonstrated in man (57). Much of the research has involved the supplementation of vitamin E deficient animals and the relevance of these studies to the human condition is questionable, as the majority of the human population is not deficient in vitamin E. A more relevant question is therefore, whether supplementation with vitamin E confers increased resistance against ozone toxicity.

Biochemical Consequences of Ozone Acting Through Free Radical Mechanisms

Under normal circumstances, the lung is protected from free radicals as a consequence of its extensive antioxidant screen. However, when an oxidant gas such as ozone is inspired in high enough concentrations, or for a sufficient duration, lung injury will occur. This will probably occur indirectly through free radical intermediates, some of which will be detectable as specific biochemical markers of oxidant injury (58). Although free radicals are usually sufficiently reactive to damage most cell components, lipid and protein are the two cell constituents which have been studied in greatest detail. The two processes concerned are referred to as lipid peroxidation and protein oxidation, respectively.

OZONE-INDUCED LIPID PEROXIDATION

Ozone will react with all hydrocarbons, but its most rapid reaction occurs with unsaturated hydrocarbons, such as PUFAs (59). It is widely assumed that PUFAs are the primary target for ozone, whether as airway lining lipoproteins or phospholipid components of membranes, and that lipid peroxidation is the most important mechanism of ozone-induced lung injury (7).

The most rapid reaction between ozone and unsaturated hydrocarbons occurs through the process of Criegee ozonation (Fig. 3). The reaction is initiated through the non-radical mediated electrophilic

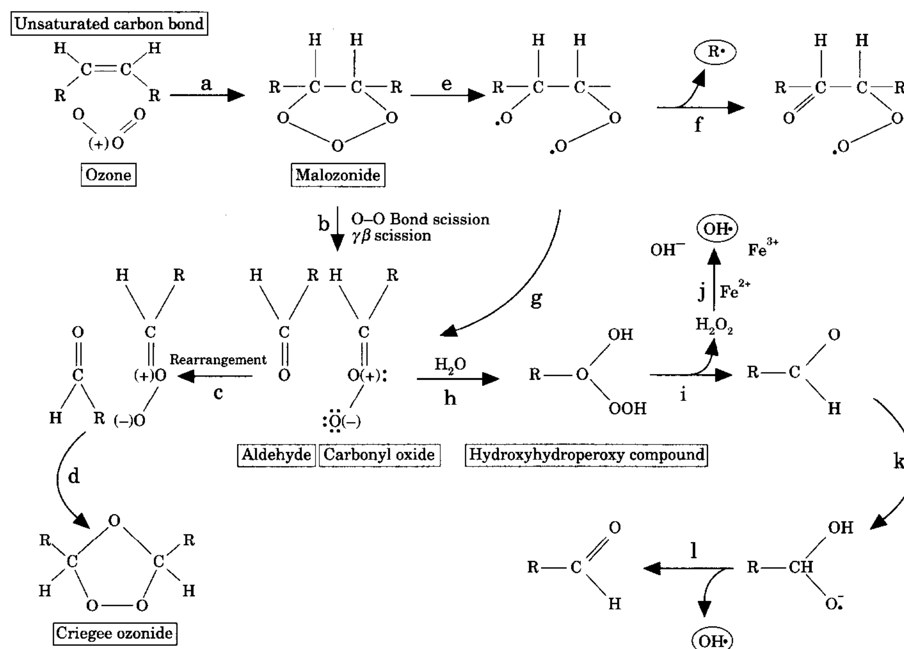

Fig. 3 Reaction scheme for ozonation of alkene bonds. Reactions (a–d) outline the classical Criegee ozonation pathway. When carbonyl oxide is formed in an aqueous environment, the reaction pathway (h–i) predominates, via the hydroxy-hydroperoxyl with the concomitant formation of H_2O_2 and OH, if free metal ions are available (j). The tri-oxygen species, malonzonide typically undergoes a $\gamma\beta$ scission to yield the carboxyloxide (b), but can still yield a diradical (e), which may then partition to give either the carboxyl oxide (g) or undergo a β scission to yield alkyl and peroxyl radicals (f).

addition of ozone to an unsaturated C-C bond which generates a tri-oxygen intermediate, 1,2,3-trioxalane, through a mechanism in which six electrons move in a concerted fashion in a cyclic transitional state. This intermediate, often termed malozonide, contains a trioxide bond which rapidly undergoes O-O bond homolysis to form a very unstable di-radical species which rapidly decomposes to liberate an aldehyde species and a carbonyl oxide. These two species then rapidly recombine in a head-to-tail orientation to form 1,2,4 trioxalane or, as it is commonly referred to, the Criegee ozonide.

It has also been demonstrated that ozone can react with reactive hydrogen atoms such as those present in acetyls to produce hydroperoxides, or in the case of aldehydes, hydrotrioxides (60) Giamalva *et al.* have hypothesized that comparable reactions might also occur at the allylic hydrogen atoms in PUFAs. If this were to occur *in vivo*, transition metal ion catalysed decomposition of the resultant hydroperoxide species would yield peroxy and alkoxy radicals, which could then act as initiators for lipid peroxidation. Though the yields of these radicals would be relatively low compared with products of Criegee ozonation, their significance as potential initiators of auto-oxidation should not be discounted due to the self-propagating nature of these reactions – i.e only small yields of free radicals are necessary to produce a large pathological outcome.

As mentioned previously, ozone is probably too reactive to transverse the epithelial lining fluid. Since unsaturated fatty acids in the lung lining fluid exist in a highly aqueous environment, ozonization would be expected to produce aldehydes and hydrogen peroxide in preference to the Criegee ozonide as a consequence of the higher affinity of the carbonyl oxide for water than for its corresponding aldehyde (61,62). This reaction would result in the production of hydroxyhydroperoxy compounds which can either react directly with transition metal ions to generate hydroxyl radicals, or undergo hydrolysis to produce hydrogen peroxide which could then decompose again in the presence of metal ions to generate further hydroxyl radicals. Experimental evidence to data has not however, supported such a pathway. Radical formation may therefore occur as a consequence of an intermediate formed prior to the carboxyloxide. The 1,2,3-trioxalane species described earlier is known to undergo a β-scission to yield a di-radical species to give carbonyl oxide. Pryor has proposed

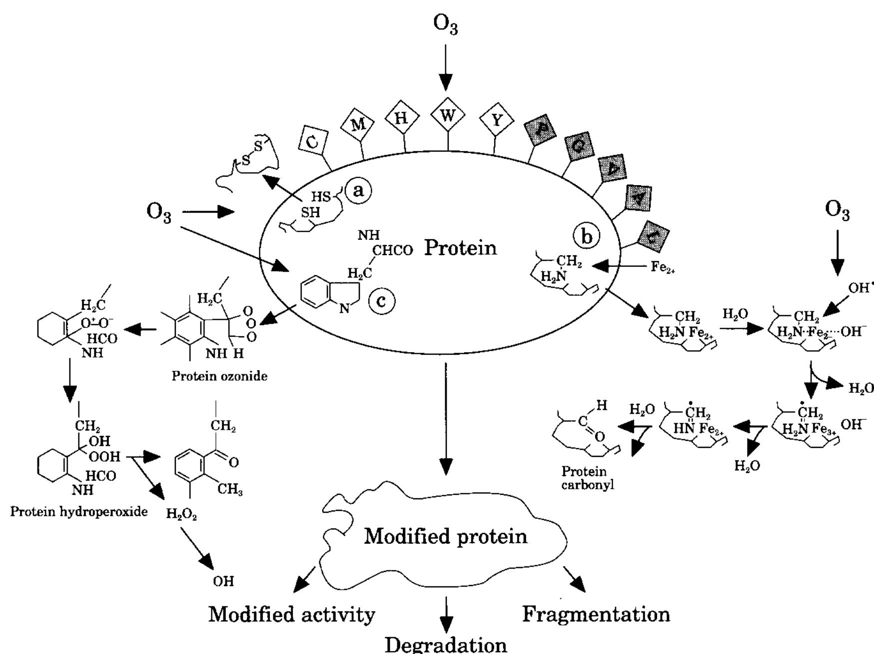

Fig. 4 Ozone-induced protein oxidation. Ozone will react directly, with high intrinsic reactivity, with a range of amino acids such as cysteine, methionine, histidine, tryptophan and tyrosine, whereas it will react with other amino acids at a much lower rate. It reacts with exposed sulphydryl groups to form disulphide bridges (a), although the exact mechanisms is unclear. It will also react with tryptophan to give protein ozonides and protein hydroperoxides (c). Protein carbonyl formation has also been demonstrated though this is probably mediated through radicals derived from ozone reacting with other biomolecules (b).

that this species can haemolyse to generate a di-radical which can eliminate a radical (Fig. 3). Whether this constitutes a minor pathway with respect to that resulting in the fixation of the carbonyl oxide directly from the trioxalaine species, or from the intermediate di-radical is not known (63).

EVIDENCE FOR OZONE-INDUCED LIPID PEROXIDATION

In a number of *in vitro* studies it has been demonstrated that ozone exposure leads to lipid peroxidation, typically measured in terms of the concentration of thiobarbituric acid reactive substances (TBARS), and that antioxidant supplementation reduces TBARS formation (64,65). In addition, lipid peroxidation products have been reported in the exhaled breath of both animals and humans, following exposure to ozone or combinations of oxidants gases (66–69). However, due to the inherent lack of sensitivity of the assays used to measure lipid peroxidation, different groups have often published contradictory results. For example, Chow and Tappel (70) found that the concentration of TBARS was elevated in rats after exposure to 0·5 or 0·7 ppm ozone, and that this was partially inhibited by vitamin E; however, subsequent studies in which vitamin E depleted rats were exposed to 0·8 ppm ozone showed no such increases (71). Other investigators have reported increased exhalation of ethane and pentane from ozone-exposed, vitamin E deficient rats (66); nevertheless comparable induction of these gases in primates, including man has not been conclusively demonstrated. Indeed in the experiments by Cross *et al.* in which plasma was exposed to 16 ppm ozone over 4 h, little lipid hydroperoxide generation could be demonstrated (20). Despite the conflicting evidence, it is generally agreed that ozone is able to induce lipid peroxidation in the lung provided that the ozone concentration is sufficiently high to compromise the antioxidant defence mechanisms, or that these defences are weakened in some way prior to the challenge.

OZONE-INDUCED PROTEIN OXIDATION

Ozone damages protein through its ability to directly, or through free radical mediated reactions, oxidize a range of functional groups including sulphydryls, amines, alcohol and aldehydes (Fig. 4). Ozone is particularly reactive towards a limited

number of amino acids including cysteine, methionine, tryptophan, and tyrosine (20,72). Studies of the reaction of ozone with amino acids in model systems have yielded a great deal of information about the relative and absolute rates of reaction between ozone and specific amino acids (19). A recent study by Pryor's group (18) using a panel of 11 tryptophan-rich proteins (albumin, carbonic anhydrase, β-casein, α-chymotrypsin, α-lactalbumin, β-lactoglobulin, lysozyme, papain, ribonuclease A, apotransferrin and trypsin) solubilized in reversed micelles has elucidated the fundamental rate constants and the influence of the protein environment on the ozone-induced oxidation of tryptophan. This study showed that: (i) the oxidation product of the protein-bound tryptophan was probably a Criegee ozonide; (ii) the ozonization of tryptophan residues resulted in the production of hydrogen peroxide, which was neither destroyed nor consumed during the reaction; and (iii) it was possible to construct a kinetic model to predict the fractional reaction of ozone with amino acid residues in proteins.

EVIDENCE OF OZONE-INDUCED PROTEIN OXIDATION

Inactivation of enzymes, such as lung prostaglandin synthetase (73), cholinesterase (74) and α1-antiproteinase (75), has been demonstrated at high (2–4 ppm) concentrations of ozone in a number of model systems. Ozone decreases the trypsin, chymotrypsin, and elastase inhibitory activities of human α1-antiproteinase inhibitor in both plasma and solutions of the pure inhibitor. Inhibition of α1-antiproteinase in the lung could contribute to the development of emphysema, thus emphasizing the potential toxicological significance of ozone-induced damage to proteins (75). In contrast to its inhibitory activity, ozone has been shown to increase proteolytic activity in homogenates of rat brain (76). The activity of calpain was elevated in brain homogenates exposed to a range of concentrations from 0–1000 ppb ozone, and the increased activity appeared to be dose-dependent. It has also been demonstrated that *in vitro* exposure of human plasma to extremely high concentrations of ozone (16 ppm) is associated with significantly elevated levels of carbonyl groups (20). In addition, isolated human albumin and creatine kinase were shown to be oxidized much more rapidly than serum proteins, indicating that the antioxidant status of the environment is an important determinant of the susceptibility of proteins to oxidative damage. The fact that mannitol had very little effect on carbonyl formation, either in the total plasma or isolated protein system, seems to indicate that the formation of hydroxyl radicals from ozone is an unimportant mechanism in this type of oxidative modification and that ozone oxidizes the proteins directly. Ozone has been shown to attack histidine and tyrosine residues *in vitro* (77) and it is probable that such reactions occur *in vivo* at environmentally relevant ozone concentrations. In addition, in one of the few studies to address the possible extrapulmonary effects of ozone, it was found that the heart contained an increased number of carbonyl groups after *in vivo* exposure to elevated concentrations of ozone (6).

Conclusions

Both controlled chamber studies and summer field camp studies have confirmed observations made previously in animals that exposure to ozone leads to increase airways resistance, pulmonary inflammation and increased pulmonary permeability. It is now recognized that inspired ozone will not transverse the lung lining fluids unreacted. As many of the reactions of ozone are via free radical intermediates, the initial targets for and defences against ozone are the antioxidant components of the lung lining fluid. As a result, understanding the composition and distribution of the antioxidant components of ELF is now of particular interest. Little is presently known about the regulation of this pool of extracellular pulmonary antioxidants. However, studies ongoing in our laboratories and in those of others working in this area will hopefully soon shed light on the benefits of antioxidant defences in lung lining fluids against atmospheric pollutants such as ozone.

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