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# Role of antioxidants in paraquat toxicity

Zacharias E. Suntres *

*Health Hazards Group, Biomedical Sciences Section, Defence and Civil Institute of Environmental Medicine, 1133 Sheppard Avenue West, Toronto, Ont., Canada M3M 3B9*

**Abstract**

Paraquat, a quarternary nitrogen herbicide, is a highly toxic compound for humans and animals and many cases of acute poisoning and death have been reported over the past few decades. The mechanisms of paraquat toxicity involve: the generation of the superoxide anion, which can lead to the formation of more toxic reactive oxygen species, such as hydrogen peroxide and hydroxyl radical; and the oxidation of the cellular NADPH, the major source of reducing equivalents for the intracellular reduction of paraquat, which results in the disruption of important NADPH-requiring biochemical processes. The major cause of death in paraquat poisoning is respiratory failure due to an oxidative insult to the alveolar epithelium with subsequent obliterating fibrosis. Management of paraquat poisoning has remained mostly supportive and has been directed towards the modification of the toxicokinetics of the poison. Currently, there are no true pharmacological antagonists for paraquat and there are no chelating agents capable of binding the poison in the blood or other tissues. Recognizing the fact that paraquat induces its toxic effects via oxidative stress-mediated mechanisms, innovations in the management of paraquat poisoning are directed towards the use of antioxidants. In this review, the status of antioxidants in ameliorating or treating the toxic effects produced by paraquat is presented. 

*Keywords:* Paraquat; Antioxidants; Oxidative stress; Humans; Animals

## 1. Introduction

Paraquat is a quarternary nitrogen herbicide widely used for broadleaf weed control. It is a fast-acting, non-selective compound, which destroys tissues of green plants on contact and by translocation within the plant. Paraquat exerts its herbicidal activity by interfering with the intracellular electron transfer systems in plants, thereby inhibiting reduction of NADP to NADPH during photosynthesis (Pasi, 1978). This disruption leads to the formation of superoxide anion, singlet oxygen, as well as hydroxyl and peroxyl radicals (Autor, 1977; Pasi, 1978). These reactive oxygen species (ROS) interact with the unsaturated lipids of membranes resulting in the destruction of plant organelles, inevitably leading to cell death (Dodge, 1971; Pasi, 1978).

The strong affinity for adsorption to soil particles and organic matter is one of the major advantages in introducing paraquat as a herbicide because it limits its bioavailability to plants and microorganisms. Moreover, paraquat is not mobile in most soils and the portion that does not become associated with soil particles can be

* Tel.: +1-416-635-2150; fax: +1-416-635-2104
*E-mail address:* zach.suntres@dciem.dnd.ca (Z.E. Suntres).

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decomposed to a non-toxic product by soil bacteria; thus, paraquat does not present a high risk of groundwater contamination. Ultraviolet light, sunlight and soil microorganisms can degrade paraquat to products that are less toxic than the parent compound (Pasi, 1978). However, paraquat has been demonstrated to be a highly toxic compound for humans and animals and many cases of acute poisoning and death have been reported over the past few decades (Autor, 1977; Bismuth et al., 1990; Gram, 1997; Pasi, 1978).

The high mortality rate observed following paraquat exposure has been attributed to the lack of an antidote or effective treatment to ameliorate the toxic effects of the poison. Recognizing that paraquat induces its toxic effects mainly via oxidative stress-induced mechanisms, researchers and clinicians have placed great emphasis on the use of antioxidants as a treatment modality for paraquat toxicity (Bateman, 1987; Meredith and Vale, 1987; Proudfoot et al., 1987; Vale et al., 1987).

## 2. Paraquat toxicity

The most frequent routes of exposure to paraquat, either accidentally or intentionally, in humans and animals are following ingestion or through direct skin contact. If ingested, paraquat induces a burning sensation of the mouth and throat, followed by gastrointestinal irritation, subsequently resulting in abdominal pain, loss of appetite, nausea, vomiting and diarrhea (Pasi, 1978). Direct contact with paraquat solutions or aerosol mists may cause skin burns and dermatitis (Spiewak, 2001). Paraquat splashed in the eyes can irritate, burn and cause corneal damage and scarring of the eyes. Due to its low vapour pressure and the formation of large droplets, inhalation of paraquat spray used in the open environment has not been shown to cause any significant systemic toxicity; however, inhalational exposure to paraquat in confined spaces, such as a greenhouse, is known to be associated with fatal pulmonary disease (Pasi, 1978).

Irrespective of its route of administration in mammalian systems, paraquat is rapidly distributed in most tissues, with the highest concentration found in the lungs and kidneys (Autor, 1977; Pasi, 1978). The compound accumulates slowly in the lung via an energy-dependent process (Rose et al., 1974). Excretion of paraquat, in its unchanged form, is biphasic, owing to lung accumulation and occurs largely in the urine and, to a limited extent, in the bile. Biotransformation of paraquat is, in general, poor in all species studied and the excreted compound is unchanged (Autor, 1977; Heath and Smith, 1977; Pasi, 1978).

The primary injury caused by paraquat to mammalian systems occurs in the lung, where paraquat is accumulated through a process of active transport in the Clara cells and alveolar type I and II epithelial cells (Rose et al., 1974; Autor, 1977; Heath and Smith, 1977). The paraquat-induced lung injury is morphologically characterized by an early destructive phase, in which the alveolar type I and type II epithelial cells are damaged; and a second proliferative phase defined by alveolitis, pulmonary edema and infiltration of inflammatory cells (Autor, 1977; Heath and Smith, 1977; Pasi, 1978; Bus and Gibson, 1984).

In addition to the lung, paraquat administration has been shown to injure other major organ systems, but to a lesser extent. Pathological changes have been observed in the liver, kidney and heart at high doses, but death is usually associated with respiratory insufficiency injury (Autor, 1977; Heath and Smith, 1977; Pasi, 1978; Honore et al., 1994). Thus, research for the treatment of paraquat toxicity has mainly focused on alleviating the lung injury.

### 2.1. Mechanisms of paraquat toxicity

Over the past few decades, research into the mechanisms of paraquat toxicity has identified several toxic outcomes of the redox cycling reaction: (i) the generation of the superoxide anion which can lead to the formation of more toxic reactive oxygen species, such as hydrogen peroxide and hydroxyl radical; (ii) the oxidation of the cellular NADPH, the major source of reducing equivalents for the intracellular reduction of paraquat, which results in the disruption of important NADPH-requiring biochemical processes (Autor,

1977; Heath and Smith, 1977; Pasi, 1978; Bus and Gibson, 1984; Comporti, 1989; Gram, 1997); and (iii) lipid peroxidation which results in the oxidative degeneration of cellular polyunsaturated fatty acids (Bus et al., 1975; Heath and Smith, 1977; Comporti, 1989; Gram, 1997).

The importance of oxidative stress as a mechanism of paraquat toxicity has been demonstrated in studies in plants (Dodge, 1971), bacteria (Fridovich and Hassan, 1979), in vitro and in vivo systems (Heath and Smith, 1977; Bus and Gibson, 1984; Comporti, 1989; Gram, 1997). More precisely, it has been demonstrated that the formation of the superoxide anion as the culprit of paraquat toxicity stems from the observations that bacteria or other biological systems (in vitro systems with lung or liver preparations) containing elevated levels of superoxide dismutase, an enzyme that detoxifies the superoxide anion, were resistant to paraquat toxicity. Also, the potentiation of paraquat-induced toxicity in animals exposed to high oxygen tensions further supports the potential role of molecular oxygen in mediating toxicity (Heath and Smith, 1977; Fridovich and Hassan, 1979; Bus and Gibson, 1984; Gram, 1997).

Increased oxidation of cellular NADPH from redox cycling of paraquat has also been suggested as a potential mechanism of paraquat toxicity. This observation is supported by several findings: (i) administration of paraquat decreases the NADPH content in rat lung; and (ii) the activity of pentose shunt enzymes in the lung rapidly increased in rats challenged with paraquat, suggesting an increased demand for NADPH (Fisher et al., 1975; Heath and Smith, 1977; Brigelius et al., 1981; Bus and Gibson, 1984; Comporti, 1989; Gram, 1997).

Lipid peroxidation, the oxidative degeneration of polyunsaturated fatty acids, has been suggested as a potential mechanism of paraquat toxicity in mammalian systems. This observation is consistent with the following findings from several studies: (i) exposure of animals to paraquat resulted in significant increases in lipid peroxidation (Bus et al., 1975; Heath and Smith, 1977; Comporti, 1989; Gram, 1997); (ii) animals fed vitamin E- or selenium-deficient diets, thereby diminishing their cellular antioxidant defences, were significantly more susceptible to paraquat toxicity than control animals (Bus et al., 1975; Omaye et al., 1978; Bus and Gibson, 1984); and (iii) tissues or cells which have low GSH concentrations are highly vulnerable to paraquat toxicity because GSH may play a major role in antagonizing the oxidative action of paraquat (Bus and Gibson, 1984; Nakagawa et al., 1995).

## 3. Treatment of paraquat toxicity

The major cause of death in paraquat poisoning is respiratory failure due to an oxidative insult to the alveolar epithelium with subsequent obliterating fibrosis. Since there are no known pharmacological antagonists for paraquat and there are no chelating agents capable of binding the poison in the blood or other tissues, strategies in the management of paraquat poisoning have been directed toward the modification of the toxicokinetics of the poison by either decreasing its absorption or enhancing its elimination. Such approaches are intended to prevent the accumulation of paraquat in tissues and include procedures, such as induced emesis or diarrhoea, gastric lavage, administration of oral absorbents, hemodialysis and hemoperfusion (Bismuth et al., 1982; Bateman, 1987; Meredith and Vale, 1987; Proudfoot et al., 1987; Vale et al., 1987; Hampson and Pond, 1988; Honore et al., 1994). However, these treatment methods have been disappointing and the mortality rate has remained high.

The potential for attack by endogenous and exogenous oxidants requires that cells use many different antioxidant strategies to combat any oxidant-induced cellular damage. Such protective measures include: (i) those aimed at preventing the generation and distribution of ROS (the effective control of iron distribution and the destruction of peroxides by catalase or by glutathione peroxidase are included in this category); (ii) those aimed at reactive metabolite scavenging including the maintenance of effective levels of antioxidants, such as vitamin E, vitamin C, β-carotene and glutathione, as well as the enzyme superoxide dismutase (SOD); and (iii) those aimed at free radical repair, particularly the maintenance of effective levels of

glutathione (Sies, 1987; Evans and Halliwell, 2001).

### 3.1. Superoxide dismutase

Under normal circumstances, formation of superoxide anion produced by paraquat and other chemicals is kept under control by the SOD enzymes. These include: the copper-zinc SOD, which is the primary species in the cytoplasm; the manganese SOD, which is the primary species in the mitochondria; and the extracellular SOD, which is the major form of SOD in extracellular fluids (Fridovich, 1975).

The use of SOD as a treatment to ameliorate paraquat-induced injuries has produced variable results. Exogenously-administered SOD conferred protection in young rats that had been challenged with paraquat (Autor, 1974). Also, in adult rats, SOD reduced the mortality to paraquat challenge from $\approx$80 to 45% over a 28-day period (Wasserman and Block, 1978). The protective effect of SOD against paraquat toxicity has been attributed to its ability to scavenge the superoxide anion, generated from the redox cycling of paraquat.

In contrast, the results from most studies in which SOD had been employed as an antioxidant treatment for paraquat toxicity demonstrate that when SOD was administered by continuous intravenous infusion, it failed to ameliorate the toxic effects of the herbicide (Block, 1979). Furthermore, SOD failed to protect against paraquat toxicity in vitamin E-deficient animals raising the possibility that, in the absence of vitamin E, it is possible that the peroxidative chain reactions may be triggered and sustained by small amounts of superoxide anion escaping detoxification by SOD (Block, 1979). It has been reported that the lack of effectiveness by SOD in protecting against paraquat toxicity can be attributed to its physicochemical properties; this enzyme cannot enter the target cell membrane because of its high molecular mass (which prevents intracellular transport) or its charge (which prevents its adherence to targets) (Freeman et al., 1985; Patel and Day, 1999; Muzykantov, 2001)

More recently, in order to circumvent these problems, investigators have used low-molecular-weight metalloporphyrin SOD mimetics or liposomal encapsulated SOD for the purpose of successfully treating oxidative stress-induced injuries. More precisely, Day and Crapo (1996) employed the low-molecular-weight metalloporphyrin superoxide mimetic, tetrakis-(4-benzoic acid) porphyrin (MnTBAP), to protect mice against paraquat-induced lung injury. This SOD mimetic has been demonstrated to penetrate cell membranes, retain its activity intracellularly and also protect endothelial cells against intracellular paraquat-induced injury in vitro (Day et al., 1995; Patel and Day, 1999). Although no studies have examined the role of liposomal encapsulated SOD against paraquat-induced injuries, it has been reported that elevated enzyme levels in the lungs of rats treated with liposome-encapsulated SOD were accompanied by a significant improvement in lung assessment and survival rates after exposure to hyperoxia (Freeman et al., 1985; Padmanabhan et al., 1985) or bleomycin (Ledwozyw, 1991).

### 3.2. Antioxidant vitamins

#### 3.2.1. Ascorbic acid (Vitamin C)

Ascorbic acid, a water-soluble vitamin, is effective in scavenging free radicals, including hydroxyl radical, aqueous peroxyl radicals and superoxide anion. Ascorbic acid acts as a two-electron reducing agent and confers protection by contributing an electron to reduce free radicals, thus neutralizing these compounds in the extracellular aqueous environment prior to their reaction with biological molecules (Carr and Frei, 1999; Evans and Halliwell, 2001). High concentrations of ascorbic acid are found naturally in the fluid of the lung to protect against free radicals generated by toxic chemicals in air, such as ozone, sulfur dioxide, metal fumes and cigarette smoke (Menzel, 1994; Carr and Frei, 1999). Moreover, the antioxidant potential of ascorbic acid is not only attributed to its ability to quench reactive oxygen species, but also to its ability to regenerate other small molecule antioxidants, such as α-tocopherol, glutathione and β-carotene (Halliwell, 1996; Carr and Frei, 1999; Evans and Halliwell, 2001).

Intravenously-administered vitamin C shortly prior to paraquat challenge protected against

tissue damage as evidenced by a reduction in expiratory ethane, a reliable index of oxidative damage (volatile hydrocarbons, such as ethane, are produced from the damaged tissue and reflect the extent of peroxidized unsaturated fatty acids) (Kang et al., 1998). In a study examining the embryotoxicity of paraquat by utilizing the frog embryo teratogenesis assay-xenopus (FETAX) bioassay, it was shown that pretreatment with ascorbic acid protected the embryos (Vismara et al., 2001). The mechanism for such a protective effect has been attributed to the ability of vitamin C to quench radicals generated by the redox cycling of paraquat before they attacked other biomolecules.

Although prior administration of ascorbic acid confers protection against paraquat toxicity, the use of ascorbic acid in treating paraquat-induced tissue injuries has resulted in unfavorable consequences. Apparently, ascorbic acid can accelerate the generation of hydroxyl radicals by accelerating the redox cycling of free transition metal ions (i.e. $Fe^{+3}/Fe^{+2}$) in the aqueous phase (Buettner and Jurkiewicz, 1996; Halliwell, 1996; Carr and Frei, 1999; Evans and Halliwell, 2001). Results show that, during extensive cellular damage, transition metals are released into the aqueous phase (Kohen and Chevion, 1985; Halliwell, 1996). Ascorbic acid, given at a time when the extensive tissue damage induced by paraquat is in progress, aggravates the oxidative damage (Kang et al., 1998). The exacerbation of the oxidative damage following the interaction of transition metals with ascorbic acid during the progressive stages of paraquat toxicity, was significantly reduced by pretreating these animals with desferoxamine, a chelator that tightly binds the ferric iron just prior to paraquat administration (Kang et al., 1998).

### 3.2.2. Vitamin E (α-Tocopherol)

Vitamin E is a lipid-soluble vitamin that exerts its antioxidant effects by scavenging free radicals and stabilizing membranes containing polyunsaturated fatty acids (Witting, 1980; Burton, 1994). Results from in vivo studies have demonstrated that vitamin E administered in large doses over a prolonged period of time confers protection against oxidant-induced tissue damage (Roehm et al., 1971; Bucher and Roberts, 1981; Knight and Roberts, 1985; Chow, 1991).

The role of vitamin E in paraquat toxicity was demonstrated in several studies where deficiency of vitamin E potentiated the development of acute paraquat toxicity in animals. It was shown that vitamin E deficiency shortened and decreased survival, worsened histologic lung damage in rats (Block, 1979) and significantly reduced the LD50 in mice (Bus et al., 1975) exposed to paraquat. Moreover, the potentiation of acute paraquat toxicity by vitamin E deficiency was reversed by administration of vitamin E (Block, 1979). Although the mechanism(s) by which vitamin E protects against paraquat toxicity is not understood, it may be attributed to its antioxidant properties in preventing lipid peroxidation or inhibiting the generation of superoxide anion and its toxicity.

Although vitamin E confers protection against paraquat-induced injuries in vitamin E-deficient animals, normal animals receive little benefit from additional pharmacologic supplementation with vitamin E. In a study investigating the presence of lipid peroxidation as a potential marker of subacute toxic reaction, it was shown that vitamin E supplementation to humans (100–900 mg per day) was ineffective in protecting against paraquat poisoning and did not affect the levels of lipid peroxidation (Yasaka et al., 1986). Moreover, administration of vitamin E either 30 min after paraquat challenge followed by a second injection 24 h later, or 2 h before paraquat challenge followed by a second injection 26 h later, did not alter the acute mortality nor reduce the characteristic pathological lung changes observed at death (Redetzki et al., 1980). Similar findings have been reported by other investigators who found that the administration of vitamin E in normal animals was not effective in ameliorating injuries induced by oxidants (Ramazzotto and Engstrom, 1975; Stephens et al., 1983; Warren et al., 1988).

The failure of vitamin E to protect normal animals against paraquat and other oxidants is unclear at the present time. It has been suggested that this ineffectiveness might be related to the solubility of vitamin E, since lipid-soluble antioxidants take too long to diffuse through cellular

membranes. Moreover, when tocopheryl acetate is administered to animals, the rate-limiting step in the bioavailability of the physiologically free tocopherol is the hydrolysis rate of the acetate ester (Newmark et al., 1975). To overcome this major limitation in patients requiring emergency treatment, water-soluble analogs of α-tocopherol, which can be given safely intravenously (Petty et al., 1990), or liposomal α-tocopherol preparations (Suntres et al., 1992, 1993; Suntres and Shek, 1995c) might offer a better treatment effect.

### 3.3. Melatonin

Treatment of paraquat challenged rats with melatonin reduced mortality and resulted in marked protection against paraquat-induced liver and lung injuries as evidenced by the reversal of lipid peroxidation and glutathione depletion (Melchiorri et al., 1995, 1996). In more recent studies, the role of melatonin in alleviating paraquat-induced toxic effects, other than the major organ injuries, such as its genotoxic effects, has been investigated (Reiter, 1999). It has been shown that paraquat mediates its genotoxic effect, partly via its capacity to generate ROS. An increase in the frequency of sister-chromatid exchanges and chromosomal aberrations is apparent in paraquat-treated Chinese hamster lung cells (Tanaka and Amano, 1989). Paraquat possesses a dose-dependent mutagenic activity in several eukaryotic systems, including human lymphocyte cultures (Salam et al., 1993). Results from another study demonstrated that paraquat was able to induce the formation of micronuclei, commonly used to assess chromosomal damage, in polychromatic erythrocytes (PCE), both in the bone marrow and in the peripheral blood of mice, a treatment effect attributed to the generation of ROS (Melchiorri et al., 1998). Administration of melatonin to these mice conferred protection against the paraquat-induced micronuclei and this effect was attributed to the antioxidant properties of the pineal secretory product (Melchiorri et al., 1998).

Melatonin exerts its antioxidant effects by scavenging hydroxyl and peroxyl radicals and possibly singlet oxygen (Cagnoli et al., 1995; Reiter et al., 1995, 1997). Furthermore, it has been shown that melatonin stimulates the endogenous antioxidant enzyme glutathione peroxidase and thus contributes to the enhanced cellular defenses against oxidative stress (Reiter et al., 1995; Pablos et al., 1996) and stabilizes cell membranes, which then become more resistant to oxidative attack (Garcia et al., 1998).

### 3.4. Iron chelators

The importance of iron and other transition metals in oxygen-radical-generated damage in several conditions, including paraquat exposure, has been demonstrated by the beneficial effect of the transition metal-chelators to protect against these injuries (Kohen and Chevion, 1985; Hershko and Weatherall, 1988). Results from both in vitro and in vivo studies have shown that iron chelation can prevent paraquat toxicity (Kohen and Chevion, 1985; Van Asbeck et al., 1989; Van der Wal et al., 1990, 1992), a treatment effect that also depends on the lipophilicity of the chelating agents (Van der Wal et al., 1992). The administration of desferoxamine (DFO) by continuous intravenous infusion to vitamin E-deficient rats significantly reduced mortality produced by paraquat (Van Asbeck et al., 1989). It has been shown that DFO can exert its protective effects, not only by inhibiting the paraquat-induced generation of hydroxyl radicals, but also by blocking the uptake of paraquat by the alveolar type II cells (Van der Wal et al., 1990). Administration of more lipophilic chelating agents, such as hydroxypyridin-4-one (CP 51), also increased the survival of paraquat-challenged rats with a normal vitamin E status. Moreover, the protective effect of CP51 was also demonstrated in vitro experiments where CP51 prevented the paraquat-induced lysis of alveolar type II cells (Van der Wal et al., 1992). Although experimentation with iron chelators against paraquat-induced toxicity seems promising, iron chelation therapy in human poisoning remains to be established.

### 3.5. Low molecular weight thiol-containing antioxidants

#### 3.5.1. Glutathione

Glutathione (GSH) is the most abundant non-protein thiol in living organisms and it plays a crucial role in intracellular protection against toxic compounds, such as ROS and other free radicals (Anderson, 1997; Anderson and Luo, 1998). Glutathione can function as a nucleophile to form conjugates with many xenobiotic compounds and/or their metabolites and can also serve as a reductant in the metabolism of hydrogen peroxide and other organic hydroperoxides, a reaction catalyzed by glutathione peroxidases found in cytosols and mitochondria of various cells (Anderson, 1997; Anderson and Luo, 1998; Lu, 1999; Rahman et al., 1999; Deneke, 2000).

Several studies have demonstrated that GSH content in the lung, as well as in other tissues, is decreased in certain pathological conditions, such as hyperoxia, ischemia/reperfusion and following administration of oxidants or electrophiles. Depletion of GSH from the cellular pool, either as a result of a disease condition or an experimental administration of thiol depleters, renders cells and living organisms more susceptible to the effects of oxidants. Therefore, GSH is important in conferring protection and preserving the integrity of the living organism (Anderson, 1997; Anderson and Luo, 1998; Rahman et al., 1999).

Although in vitro studies have shown that alveolar type II cells can supplement endogenous synthesis of GSH with uptake of exogenous GSH to protect against paraquat-induced injury (Hagen et al., 1986; Brown et al., 1992), the antioxidant effectiveness of exogenously administered GSH for the treatment of pulmonary injuries against paraquat or other oxidants has been hindered by its rapid hydrolysis in the circulation and its inability to cross cell membranes (Meister and Anderson, 1983; Jurima-Romet et al., 1990; Smith et al., 1992). Results from several studies have demonstrated that the instillation of free GSH fail to protect the lung against oxidant insults and this ineffective treatment effect is caused by its rapid removal from the lung (Jurima-Romet et al., 1990; Smith et al., 1992; Suntres and Shek, 1994, 1996c). Only 1–2% of the dose administered is recovered in the lung 24 h post-treatment (Jurima-Romet et al., 1990; Smith et al., 1992; Suntres and Shek, 1994). Supplementation of animals with GSH precursors over prolonged periods of treatment results in increases in the intracellular GSH pool and decreases in the susceptibility of the biological system to oxidant-induced tissue injury (Anderson and Luo, 1998; Kelly, 1998; Deneke, 2000).

#### 3.5.2. *N*-Acetylcysteine

*N*-Acetylcysteine (NAC), the acetylated variant of the amino acid L-cysteine, is an excellent source of sulfhydryl (SH) groups. NAC is converted in the body into metabolites capable of stimulating GSH synthesis, promoting detoxification and acting directly as free radical scavenger (Moldeus et al., 1986; Kelly, 1998; Anderson and Luo, 1998; Deneke, 2000). It has been shown that the administration of NAC prior to paraquat challenge protects against paraquat toxicity in rats; animals pretreated with NAC displayed less edema and cellular infiltration in the lung than control animals without NAC pretreatment (Wegener et al., 1988). Also, the incubation of NAC with alveolar type II cells, which are known to be specific targets of paraquat toxicity in vivo, enhanced the glutathione content of these cells and consequently prevented the paraquat-induced cytotoxicity (Hoffer et al., 1996). In another study, the administration of NAC to paraquat-challenged animals delayed the paraquat-induced release of chemoattractants for neutrophils in the broncheoalveolar lavage fluid and significantly reduced the infiltration of inflammatory cells suggesting that NAC can confer its protective effect by delaying inflammation (Hoffer et al., 1993, 1997). Exposure of human alveolar cells in vitro to paraquat produced apoptotic cell death, perhaps via oxidative stress mechanisms and this toxic effect was inhibited by NAC, a treatment effect attributed to the direct scavenging action of the sulphydryl group of NAC (Cappelletti et al., 1998).

#### 3.5.3. Metallothionein

Metallothionein (MT) is a metal-binding protein of low molecular weight, containing cysteine as

one-third of its total amino acids (Moffatt and Denizeau, 1997; Deneke, 2000). This protein has been shown to be an efficient scavenger of reactive oxygen species, such as superoxide anion and hydroxyl radicals (Merker et al., 2000; Miles et al., 2000). Synthesis of MT can be induced by essential metals, such as zinc (Zn) and copper. Induction of metallothionein in the lungs of mice after Zn administration protected against the lethality and pulmonary toxicity of paraquat (Satoh et al., 1992). The protective role of MT in paraquat toxicity has also been demonstrated in transgenic mice deficient in MT genes. In these experiments, it was shown that tissues in MT-null mice were more susceptible to paraquat-induced oxidative stress than normal mice, as evidenced by increases in lipid peroxidation (Sato et al., 1996). Similarly, Lazo et al. (1995) showed that embryonic cells derived from MT-null mice were susceptible to ROS produced by paraquat. A major reason for the increases in the susceptibility of these tissues to paraquat has been attributed to the lower basal levels of non-protein thiols. It has been demonstrated that non-protein thiols, including MT and GSH, could be the first line of defence against oxidative stress-induced injuries (Deneke, 2000).

### 3.6. Liposomal antioxidants

As discussed previously, agents with antioxidant activity, such as SOD, catalase, GSH, ascorbic acid and vitamin E, have been used in treating paraquat-exposed humans and animals with limited or no success. The failure of these antioxidants to seriously modify the toxicity of the herbicide has been attributed mostly to their inability to cross cell membrane barriers and/or to their rapid clearance from cells. Recently, it has been demonstrated that the encapsulation of antioxidants in liposomes improves their therapeutic potential against oxidant-induced lung damage, including paraquat pulmonary toxicity because liposomes presumably facilitate intracellular delivery and prolong the retention time of entrapped agents inside the cell (Shek et al., 1994; Allen, 1998; Langner and Kral, 1999).

Liposomes are phospholipid vesicles composed of lipid bilayers enclosing an aqueous compartment. Hydrophilic molecules can be encapsulated in the aqueous spaces and lipophilic molecules can be incorporated into the lipid bilayers. Liposomes, in addition to their use as artificial membrane systems, are used for the selective delivery of antioxidants and other therapeutic drugs to different tissues in sufficient concentrations to be effective in ameliorating tissue injuries. The relative ease in incorporating hydrophilic and lipophilic therapeutic agents in liposomes; the possibility of directly delivering liposomes to an accessible body site, such as the lung; and the relative non-immunogenicity and low toxicity of liposomes, have rendered the liposomal system highly attractive for drug delivery (Shek et al., 1994; Allen, 1998; Langner and Kral, 1999).

#### 3.6.1. Liposome-entrapped GSH

Intratracheal instillation of liposome-encapsulated GSH significantly prolongs the half-life of the antioxidant in the lung. Liposome encapsulation altered the pulmonary retention of GSH, with 18 and 10% of the dose administered remaining in the lung 24 and 48 h post-treatment, whereas only 1–2% of the dose administered as free GSH was recovered in the lung 24 h post-treatment (Smith et al., 1992; Suntres and Shek, 1994).

The improved antioxidant effectiveness of liposomal-entrapped GSH over free GSH has been demonstrated in paraquat- and hyperoxia-induced acute lung injuries. Suntres and Shek (1996c) showed that intratracheal instillation of liposome-entrapped GSH yielded better protection than free GSH against paraquat-induced lung injury. In another study, Smith et al. (1992) showed that intratracheal instillation of liposomal GSH faired better in protecting against hyperoxia-induced lung injury. In both studies, the improved protection conferred by the liposomal formulation was attributed to the prolonged retention of liposomes in the lung, thus allowing a slow release of its GSH content.

#### 3.6.2. α-Tocopherol liposomes

After a single intratracheal instillation of α-tocopherol liposomes, high levels of [$^3$H]-labelled

α-tocopherol were recovered in the lung and its retention in the lung was prolonged (Suntres et al., 1993). Analysis of lung homogenates, isolated 24 h after the intratracheal instillation of radiolabelled α-tocopherol liposomes revealed that 79% of the label remained in the lung, presumably still associated with liposomes or their fragments (Suntres et al., 1993). In contrast, the recovery of radioactivity from rat lungs after parenteral administration of [$^3$H] α-tocopherol was 4.8 and 3.6% of total radioactivity, at 0.5 h and 48 h post-treatment, respectively, while 0.4% of initial α-tocopherol was found in the lung 12 h after intragastric administration (Gallo-Torres, 1980).

It has been shown that the intratracheal instillation of α-tocopherol liposomes alleviated most of the pulmonary toxicity induced by paraquat as well as by other oxidants, such as bleomycin, phorbol myristate acetate and lipopolysaccharide (Suntres et al., 1992; Suntres and Shek, 1995a,b,c, 1996a,b, 1997, 1998). Other studies have also shown the important role of α-tocopherol in modulating oxidant-induced cellular injury, permitting cells with high levels of the antioxidant to become more resistant to oxidative insults. It has been demonstrated that the administration of vitamin E prior to an oxidative challenge reduces the level of lipid peroxidation in the lung and other tissues and improves survival. In contrast to our studies in which the intratracheal instillation of α-tocopherol liposomes conferred a significant protective effect, the administration of non-liposomal α-tocopherol to animals by the oral or parenteral routes offered limited or no protection against oxidant-induced lung damage (Ramazzotto and Engstrom, 1975; Redetzki et al., 1980; Stephens et al., 1983; Yasaka et al., 1986; Warren et al., 1988). Results from studies examining the organ uptake and distribution of α-tocopherol have shown that the amount of antioxidant recovered from the lungs of animals after oral or parenteral administration was $<40$ μg/g lung tissue (Gallo-Torres, 1980; Knight and Roberts, 1985). The intratracheal instillation of α-tocopherol liposomes, however, can achieve a substantially higher antioxidant level in the lung, $\approx 1$ mg/g lung weight (Suntres et al., 1993). It is evident that the apparent difference in the α-tocopherol effect between our study and other studies may well be due to a difference in α-tocopherol concentration delivered to the lung.

#### 3.6.3. *Bifunctional antioxidant liposomes*

It has been shown that the administration of liposomes containing more than one antioxidant is more advantageous in ameliorating paraquat-induced lung injury (Suntres and Shek, 1996c). Other studies have also shown that the antioxidant effect of liposomal formulations containing SOD and catalase is more effective than those containing a single antioxidant (Turrens et al., 1984; Freeman et al., 1985).

The therapeutic efficacy of an antioxidant liposome formulation, containing a lipophilic antioxidant, α-tocopherol, can be improved by encapsulating another antioxidant, such as GSH, in the same liposome preparation. These bifunctional liposomes, containing both α-tocopherol and GSH, have been determined to be more effective in protecting against oxidant-induced lipid peroxidation than those containing either α-tocopherol or GSH alone. It has been shown that α-tocopherol can exert an antioxidant effect by scavenging free radicals and stabilizing biological membranes while GSH, in addition to its ability to act as a free radical scavenger, can also regenerate α-tocopherol from its oxidized form (Suntres and Shek, 1996c).

### 3.7. *Oils and other fatty acids*

The role of nutrients in modulating paraquat toxicity in experimental animals has also been investigated, but not as extensively as for antioxidants. It was noted that an intramuscular injection of commercial corn oil, which was used for the administration of lipophilic anti-inflammatory agents, dramatically reduced the lethality of a single, oral dose of paraquat in mice from 70 to 50%. Similarly, the injection of other fresh commercial vegetable oils of different ratios of unsaturated to saturated fat (sunflower 10:2, corn 8:2, peanut 5:4, olive 1:2 and coconut 0:12 oils) as well as fish oils (cod liver and menhaden oils) also reduced paraquat lethality (Fritz et al., 1994). The protective effect conferred by these oils is not

clear, but it does not appear to be due to their vitamin E content or due to alteration in the absorption or distribution of paraquat (Fritz et al., 1994). On the other hand, the loading of hepatocytes with PUFA (α-linolenic acid) underwent lipid peroxidation to a greater extent and at much lower paraquat concentrations than normal unloaded hepatocytes (Sugihara et al., 1995). It has been demonstrated that an increase in monosaturated fatty acids or a reduction in polyunsaturated fatty acids in lipid membranes decreases the susceptibility of membranes to oxidant attack (Horton and Fairhurst, 1987; Fritz et al., 1994; Sugihara et al., 1995).

Paraquat has been demonstrated to be a highly toxic compound for humans and animals. Treatment of paraquat poisoning has remained mostly supportive and management of paraquat poisoning has been directed towards the modification of the toxicokinetics of the poison. In the past few years, innovations in the management of paraquat poisoning have also been directed toward the use of antioxidants, since paraquat induces its toxic effect via oxidative stress-mediated mechanisms. Most of the antioxidants used in treating paraquat-exposed humans and animals have failed to modify the toxicity of the herbicide and this treatment effect has been largely attributed to their inability to cross cell membrane barriers and/or their rapid clearance from cells. More recently, it has been demonstrated that the use of liposomal antioxidants or low-molecular weight SOD mimetics result in increases in their therapeutic potential against oxidant-induced lung damage, including paraquat pulmonary toxicity because they presumably facilitate intracellular delivery. Moreover, the route of administration might also improve the efficacy of antioxidants in the treatment of paraquat poisoning since direct delivery of antioxidants to the lung appears to confer a more effective protection against pulmonary injuries.

Much remains to be learned about the role of intracellular and extracellular antioxidants in paraquat toxicity and there appears to be no single strategy that can improve the outcome of paraquat poisoning. The ideal antioxidant treatment for paraquat poisoning should involve intracellular and extracellular protection against superoxide anion, hydrogen peroxide, hydroxyl radical and membrane lipid peroxidation. Thus, further research is warranted to determine the effects of a combination of antioxidants in the treatment of paraquat poisoning.

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