Therapeutic Uses of Antioxidant Liposomes

William L. Stone,[1,] and Milton Smith[2]*

Abstract

This review will focus on the therapeutic uses of antioxidant liposomes. Antioxidant liposomes have a unique ability to deliver both lipid- and water-soluble antioxidants to tissues. This review will detail the varieties of antioxidants which have been incorporated into liposomes, their modes of administration, and the clinical conditions in which antioxidant liposomes could play an important therapeutic role. Antioxidant liposomes should be particularly useful for treating diseases or conditions in which oxidative stress plays a significant pathophysiological role because this technology has been shown to suppress oxidative stress. These diseases and conditions include cancer, trauma, irradiation, retinotherapy or prematurity, respiratory distress syndrome, chemical weapon exposure, and pulmonary infections.

Index Entries: Antioxidants; α-tocopherol; γ-tocopherol; liposomes; respiratory distress syndrome pulmonary infections.

1. Introduction

This review focuses on the use of antioxidant liposomes in the general area of free radical biology and medicine. The term *antioxidant liposome* is relatively new and refers to liposomes containing lipid-soluble chemical antioxidants, water-soluble chemical antioxidants, enzymatic antioxidants, or combinations of these various antioxidants. The role of antioxidants in health and disease has been extensively discussed, and many excellent reviews and books are available ***(1–6)***.

Antioxidant liposomes hold great promise in the treatment of many diseases in which oxidative stress plays a prominent role. Oxidative stress is a physiological condition in which the production of damaging free radicals exceeds the in vivo capacity of antioxidant protection mechanisms to prevent pathophysiology. Free radicals are molecules with unpaired electrons, which are often highly reactive and damaging to biological systems. The biological membranes of subcellular organelles are major sites for free radical damage but proteins and deoxyribonucleic acid (DNA) are also significant targets. Moreover, free radicals can alter cellular signal-transduction pathways and stimulate the synthesis of inflammatory cytokines ***(7–9)***. Oxygen radicals and other reactive oxygen species (ROS) arise from the single electron reductions of oxygen.

$$O_2 + e- O_2^{*-} \quad (1)$$

$$O_2^{*-} + e^- + 2H+ \rightarrow H_2O_2 \quad (2)$$

$$H_2O_2 + e^- + H+ \rightarrow H_2O + OH^* \quad (3)$$

$$OH^* + e^- + H+ \rightarrow H_2O \quad (4)$$

$$O_2^{*-} + NO \rightarrow ONOO^- \quad (5)$$

In addition, the superoxide radical (O2*) can react rapidly with nitric oxide to yield peroxynitrite as shown in **Eq. 5**. Peroxynitrite is a reactive nitrogen oxide species (RNOS) that can also cause damage to deoxyribonucleic acid (DNA), proteins, and lipid–protein complexes (i.e., biomembranes and lipoproteins). Moreover, ONOO– is likely to be generated during inflammation and the killing of bacteria. Free radicals are generated in both the aqueous and lipid compartments of cells, and to minimize their damaging effects requires both lipid- and water-soluble antioxidants. Nevertheless, the

*Author to whom all correspondence and reprint requests should be addressed: [1]Department of Pediatrics, East Tennessee State University, Johnson City, TN, 37614. E-mail:stone@etsu.edu; [2]Amaox Ltd.,Lawton, MI, 49605.

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potential clinical use of such bifunctional liposomes has been extremely limited ***(10)***.

A primary use of antioxidant liposomes has been to define the molecular mechanism of action for various antioxidants or pro-oxidants ***(11–21)***. Antioxidants, such as butylated hydroxytoluene (BHT) and α-tocopherol (α-TOH), have also been used to prevent the oxidation of unsaturated fatty acid moieties in the phospholipids of liposomes during storage ***(22)*** or sonication ***(23)***. This chapter, however, focuses on the potential therapeutic uses of antioxidant liposomes. This is a rapidly evolving area of medical research that has not been extensively reviewed. Most of the research to date has been accomplished using in vitro cell culture systems or animal models. Very few clinical trials have been attempted, yet obvious medical situations exist (e.g., protection against influenza infection and adult respiratory distress syndrome as discussed later) in which antioxidant liposomes have enormous health-related significance. The preparation of antioxidant liposomes that can be targeted to specific sites in the body is also a promising area but awaits further research. Most chemical antioxidants are phytoceuticals whose properties have already been extensively studied and are generally regarded as nontoxic and safe for human consumption ***(24)***.

In the following sections, we first review the varieties of antioxidants that have either been used in antioxidant liposomes or hold the promise of such utilization. We then focus on issues relating to the modes of administration and lastly describe the clinical uses of antioxidant liposomes for diseases in which oxidative stress plays a major role. Major emphasis is placed on the use of antioxidant liposomes for pulmonary diseases.

2. Lipid-Soluble Antioxidants

The lipid-soluble antioxidants that can be incorporated into liposomes include vitamin E (TOHs and tocotrienols) ***(25)***, ubiquinones ***(26)***, retinoids ***(27–29)***, carotenoids (e.g., lutein, β-carotene, lycopene, astaxanthin, and peridinin ***[30–33]***, lipid-soluble flavonoids (e.g., quercetin, hesperetin, naringenin) ***(34)***, soy isoflavones (genistein and daidzein) ***(35)***, tamoxifen ***(36,37)***, as well as synthetic lipid-soluble antioxidants such as BHT, tertiary-butyl hydroquinone (TBHQ), and probucol. Nitric oxide can also be incorporated into liposomes where it can inhibit free radical-mediated cholesterol peroxidation ***(38)***.

TOHs can readily be incorporated into both monolayers of unilamellar liposomes in a monomeric form ***(25)***. Furthermore, TOH in liposomes can undergo spontaneous intermembrane transfer to an acceptor membrane without the fusion of the TOH liposome ***(25)***. This intermembrane transfer is more pronounced when the TOH liposome contains polyunsaturated fatty acids ***(25)***. RRR-α-TOH and RRR-α-tocotrienol are forms of vitamin E that have the same aromatic chromanol head group but differ in the structure of their hydrocarbon tails. RRR-α-tocotrienol is, however, a better peroxyl radical scavenger than RRR-α-TOH in phosphatidylcholine liposomes ***(39)***.

β-Carotene (a carotinoid) can be incorporated into liposomes to a maximum of about 0.5*M* (based on phospholipid), whereas TOH can be incorporated at levels as high as 30 mol%. The ability of β-carotene in liposomes to inhibit free radical-mediated lipid peroxidation appears, however, to be much lower than that of α-TOH ***(40)***. Indeed, Chen and Djuric ***(41)*** found that carotenoid-containing liposomes were very sensitive to degradation by free radicals generated from iron and 2,2'-azobis(2-amidinopropane) dihydrochloride but were not protective against lipid peroxidation. β-Carotene at 0.45 mol% (of phospholipid) is, however, a more powerful inhibitor of singlet oxygen-mediated lipid peroxidation than α-TOH at 0.45 mol% ***(42)***. α-TOH at 4.5*M* is, however, also effective at inhibiting both free radical lipid peroxidation as well as singlet oxygen mediated lipid peroxidation ***(42)***. Singlet oxygen can be generated by photosensitizes and this ROS may contribute to light-induced skin toxicity as well as the aging of skin. Antioxidant liposomes have been proposed as a tool for optimizing photochemotherapy ***(43)***.

The lipids used in the preparation of antioxidant liposomes also provide an opportunity to introduce antioxidant capacity into liposomes. For example, plasmalogens (1-alkenyl, 2-acyl-) phospholipids

are thought to have antioxidant properties ***(44,45)***. Liposomes constructed with ethanolamine plasmalogen inhibit both iron- and copper-dependent peroxidation in the presence of preformed lipid hydroperoxides ***(46)***. Sindelar et al. ***(47)*** have show that plasmalogens protect polyunsaturated fatty acids from oxidative damage and that the vinyl ether function of plasmalogens is consumed simultaneously. Koga et al. have synthesized a novel phospholipid containing a chromanol structure as its polar head group ***(18,48)***. This phosphatidyl derivative of vitamin E is almost as effective an antioxidant as α-TOH in unilamellar liposomes subjected to free radicals generated in the lipid phase. The potential therapeutic value of liposomes with antioxidant phospholipids has not been explored, but this is an obvious area for future research.

A major advantage of antioxidant liposomes is their ability to simultaneously contain (and deliver) both water- and lipid-soluble antioxidants ***(32)***. This is particularly important in the case of liposomes with both TOH and ascorbate (Asc) because it has been demonstrated that ascorbate can regenerate TOH from the tocopheroxyl radical (TO*) ***(49)***.

$$TO^* + Asc \rightarrow TOH + Asc^* \qquad (6)$$

Junghans et al. ***(32)*** have used unilamellar liposomes to investigate the interaction of GSH with the lutein, β-carotene and lycopene in preventing lipid peroxidation. This group found that GSH and carotenoids interacted to improve the resistance of biological membranes toward lipid peroxidation, but the optimal level for protection varied between the different carotenoids ***(32)***.

3. Water-Soluble Antioxidants

The water-soluble antioxidants that can be used in antioxidant liposomes include ascorbate (vitamin C), urate, glutathione, *N*-acetylcysteine (NAC), lipoic acid (or dihydrolipoic acid, which is its reduced form), pro-cysteine, and water-soluble flavonoids (as in pycnogenol). Dihydrolipoic acid is somewhat unique because it can quench peroxyl radicals generated both in the aqueous phase and in membranes ***(50)***. Chemical antioxidants generally act by donating an electron to a free radical (thereby quenching the free radical) or by serving as a substrate for an antioxidant enzyme. Glutathione, for example, is itself an antioxidant ***(12)*** and can also function as a substrate for glutathione peroxidase, a key (selenium-containing) antioxidant enzyme that converts lipid hydroperoxides (LOOHs) or H_2O_2 into the corresponding lipid alcohols (LOHs) or H_2O. Chemical antioxidants can also be chelators of transition metal ions that catalyze lipid peroxidation reactions. Urate, which is present at very high concentrations in human plasma, is an excellent antioxidant that can both chelate transition metal ions and also quench aqueous free radicals ***(51–53)***.

4. Entrapped Antioxidant Enzymes

The application of antioxidant liposomes to problems of medical interest has primarily been with liposomes containing entrapped antioxidant enzymes. Recombinant biotechnology has provided the means to obtain large (i.e., commercial) quantities of human antioxidant enzyme, but these enzymes do not normally penetrate the plasma membrane of cells and have a short half-life when introduced into the body by intravenous injection. Turrens has reviewed the potential of antioxidant enzymes as in vivo pharmacological agents ***(54)***. The attachment of polyethylene glycol (PEG) to antioxidant enzymes increases their in vivo half-lives and their effectiveness in preventing pulmonary oxygen toxicity in rats ***(55)***. The various procedures for preparing liposomes with entrapped antioxidant enzymes have been evaluated by Aoki et al. ***(56)***. This group and others ***(57)*** have found that positively charged liposomes have a superior trapping efficiency for superoxide dismutase (which has a negative charge).

Early work by Freeman et al. ***(58)*** has shown that porcine aortic endothelial cells treated with liposomes with entrapped superoxide dismutase (SOD) liposomes can dramatically increase their cellular SOD levels and thereby protect the cells from oxygen-induced injury. In a key paper, Beckman et al. ***(59)*** found that endothelial cells treated with liposomes containing entrapped SOD and catalase (SOD + CAT liposomes) can increase

the cellular-specific activity of these enzymes by at least 40-fold within 2 h. These results are particularly important because endothelial cells are major sites for oxidative damage. Moreover, intravenous antioxidant liposomes would certainly make contact with vascular endothelial cells under in vivo conditions.

5. Modes of Administration

Antioxidant liposomes can be administered topically, intratracheally, intravenously, by inhalation in an aerosol form, subcutaneously, or by intramuscular injection. Topical administration can certainly be long term and is of considerable interest to the cosmetic industry in treating specific skin disorders such as psoriasis. α-Tocopheryl acetate in liposomes has been found to have a better dermal absorption than free α-tocopheryl acetate ***(60)***. Topical administration of antioxidant liposomes could also be useful in situations where individuals were exposed to toxic substances causing skin damage by free radical mechanisms (e.g., chemical warfare agents). Inhalation and intratracheal administration can be useful for those situations in which pulmonary tissues are subjected to oxidative stress, such as with influenza infection or inhalation of toxic substances such as paraquat, which is quaternary nitrogen herbicide ***(2**,**10)***.

Intravenous administration would primarily be limited to situations in which oxidative stress is a component of an acute trauma or disease. The intravenous use of antioxidant liposomes has the potential for rapidly increasing the plasma and tissue concentration of antioxidants far beyond what oral administration could achieve. Moreover, the proteolytic and bioselective processes of the gastrointestinal tract do not limit the types of antioxidants that can be administered via intravenous antioxidant liposomes. For example, it is known that plasma levels of α-TOH are about 10 times higher than the levels of γ-TOH despite the fact that dietary levels of γ-TOH are at least two times that of α-TOH. Nevertheless, γ-TOH has a unique chemical ability to detoxify peroxynitrite that is not shared with α-TOH ***(61)***. Peroxynitrite is a powerful oxidant formed by the reaction of nitric oxide with superoxide radicals (*see* **Eq. 5**) and may be an important mediator of acute oxidant tissue damage. It is reasonable to suspect, therefore, that medical situations could arise in which it would be desirable to rapidly increase plasma (and tissue) levels of γ-TOH. The poor bioavailability of orally administered γ-TOH makes this very difficult to accomplish. This limitation could, however, be overcome by the intravenous administration of liposomes containing γ-TOH.

Vitamin E used in oral supplements is often in the form of a tocopheryl ester such as tocopheryl acetate or tocopheryl succinate. Tocopheryl esters are not, however, absorbed and must first be acted on by intestinal esterases to liberate the unesterified TOH. It is interesting, therefore, that α-tocopheryl succinate, but not α-TOH, has been found to inhibit the activation of nuclear factor κB (NFκB) in cultured macrophages ***(62)***.

NFκB is a key transcription factor that regulates the expression of many inflammatory cytokines. α-Tocopheryl succinate can be incorporated into liposomes and intravenous injection would deliver this form of vitamin E to phagocytic cells ***(63)***. Oral administration of tocopheryl succinate would not, however, be expected to deliver this form of vitamin E to cells.

It is very significant that Cu,ZnSOD liposomes administered by intravenous injection can penetrate the blood–brain barrier and significantly elevate brain levels of SOD activity within 24 h ***(64**,**65)***. Moreover, the intravenous administration of Cu,ZnSOD liposomes to rats can reduce cerebral infarction caused by ischemia ***(65)*** and also inhibit learning dysfunction caused by a low dose of total body irradiation ***(66)***. Surprisingly, intraperitoneal injection of SOD liposomes has also been found to increase the brain levels of SOD in gerbils and to inhibit ischemia/reperfusion oxidative stress ***(67)***.

A major problem with conventional liposomes is that they are recognized by the immune system as foreign substances and are rapidly removed from circulation by the phagocytic cells of the reticuloendothelial system. The Kupffer cells of the liver are the most abundant population of phagocytic cells in the body. In some circumstances,

however, the uptake of conventional liposomes by hepatic Kupffer cells can actually be an advantage. Carbon tetrachloride (CCl_4), for example, is known to induce hepatotoxicity by a free-radical-mediated mechanism. Yao et al. ***(63)*** found that intravenous administration of liposomes containing vitamin E (TOH liposomes) was very effective in decreasing mortality in mice given a lethal dose of $CC1_4$. The TOH liposomes were found to primarily accumulate in the Kupffer cells of the liver.

In recent years considerable advances have been made in the design of stealth liposomes that are not recognized by the immune system and, therefore, have a much longer half-life in circulation than conventional liposomes. Stealth technology employs liposomes with a polymer coating of polyethylene glycol-phosphatidylethanolamine (PEG liposomes). Recently, the preparation of pH-sensitive stealth liposomes has been described ***(68)***. These liposomes have a prolonged circulation in vivo and destabilize at mildly acidic pH thereby being particularly efficient at delivering a water-soluble compound into a cell's cytoplasm. The use of stealth antioxidant liposomes is very new with an increasing commercial interest in their potential therapeutic applications.

Corvo et al. ***(69)*** have studied the practical aspects of subcutaneous SOD–PEG liposomal delivery with the aim of maximizing their therapeutic activity in a rat model of chronic arthritis. Rheumatoid arthritis is an autoimmune disease affecting the joints and involving the generation of damaging ROS. Antioxidants have, therefore, been proposed as potential therapeutic agents ***(70)***. Liposome size was found be the most important factor influencing the rate and extent of drainage of liposomes from the subcutaneous injection site as well as uptake by an arthritic site. Small-sized SOD–PEG liposomes(110 nm) were much more effective at targeting the arthritic site than large-sized SOD–PEG-liposomes (450 nm) ***(70)***.

In addition to encapsulating SOD (or other antioxidant enzymes) within liposomes, it is also possible to create liposomes in which these enzymes present their enzymatic activity on the external surface of liposomes. Gaspar et al. ***(71)*** have termed these liposomes with surface exposed enzymes *enzymosomes*. This group covalently linked fatty acid chains to the accessible epsilon-amino groups of the SOD (Ac-SOD). The resulting Ac-SOD was incorporated in conventional and long-circulating liposomes (Ac-SOD liposomes), which presented SOD activity on their external surfaces ***(71)***. Enzymosomes may provide a novel therapeutic tool in which enzyme release from the aqueous liposomal compartment is not required.

The ability to target liposomes to specific tissues has been the topic of considerable research. In highly imaginative work, Galovi-Rengel et al. ***(72)*** have encapsulated SOD into mucoadhesive chitosan-coated liposomes to increase their releasing time and to facilitate their cellular penetration. Chitosan is a natural aminopolysaccharide product derived from chitin, which is found in the exoskeleton of shellfish such as shrimp or crabs. Chitosan has mucoadhesive properties that have been exploited in targeting drug delivery to mucosal tissues.

This type of antioxidant-chitosan liposome could prove useful for preventing radiation damage to the esophageal lining during chemoradiotherapy for non-small-cell lung carcinoma or for protecting the esophageal lining from mustard gas toxicity.

6. Antioxidant Liposomes and Oxidative Stress

Increasing evidence suggests that oxidative stress is an important factor in the aging process and in the etiology of many chronic diseases, such as atherosclerosis, ischemic heart disease ***(73)***, rheumatoid arthritis ***(70)***, and cancer ***(74,75)***. Schwartz et al. ***(76)*** at the Harvard School of Dental Medicine have used the hamster cheek pouch tumor model to explore the potential anticancer use of various antioxidants. This group found that β-carotene liposomes injected into the oral squamous cell carcinoma of the hamster caused a lysis of the tumor cells but not of normal cells ***(76)***. Retinoids have also been shown to be clinically effective in treating diverse premalignant and malignant conditions, such as cutaneous T-cell lymphomas, leukoplakia, squamous cell carcinomas of the skin, and basal cell carcinomas ***(77,78)***.

Several investigators have documented dramatic improvement in patients with acute promyelocytic leukemia after treatment with all-*trans*-retinoic acid ***(79–81)***. However, the side effects of oral all-*trans*-retinoic acid therapy are similar to effects seen with vitamin A: headaches, other central nervous system problems, and dryness of mucosal tissues, erythema, and desquamation of skin. When incorporated in liposomes, all-*trans*-retinoic acid-associated toxicity is markedly reduced, whereas the antitumor properties (i.e., growth inhibition and differentiation induction) of all-*trans*-retinoic acid are maintained or even enhanced ***[82,83]***). Phase I and phase II clinical studies found that plasma levels of all-*trans*-retinoic acid were maintained at high concentrations even after prolonged treatment of patients with all-*trans*-retinoic acid liposomes ***(84)***. In general, the use of retinoids is safe and induces complete remission in 80 to 90% of acute promyelocytic leukemia patients. However, chronic oral administration results in reduced plasma levels associated with disease relapse in the majority of patients; this can be circumvented by using all-*trans*-retinoic acid liposomes.

Oxidative stress also contributes to the pathology observed in acute medical problems, such as heart attack ***(73,85–88)***, respiratory distress syndrome ***(89)***, trauma ***(90)***, irradiation ***(66)***, cold injury ***(91)***, and certain types of infectious diseases such as influenza and HIV infection. Evidence suggests that trauma to the brain results in the overproduction of superoxide radicals that may contribute to edema ***(92,93)***. Antioxidant liposomes containing SOD have been used effectively to treat posttraumatic brain edema ***(92,93)*** and neurological dysfunctions in rats ***(94)***.

Retinopathy of prematurity is a leading cause of blindness in premature and low-birthweight infants who are often treated with high levels of oxygen owing to surfactant deficiency. Considerable evidence ***(95–97)*** indicates that oxidative stress is a major contributor to this disease. In an animal model, Niesman et al. ***(98)*** found that intraperitoneal administration of SOD–PEG liposomes resulted in a significant increase in retinal SOD activity and an improved tolerance to high oxygen levels. Despite the enormous health-related significance, there are no clinical trials testing the efficacy of antioxidant liposomes to treat retinopathy of prematurity.

7. Pulmonary Applications of Antioxidant Liposomes

7.1. Potential Clinical Applications

Premature children often suffer from respiratory distress syndrome because they lack the capacity to synthesize pulmonary surfactant ***(99)***. Surfactant is necessary to maintain proper expansion of the small air sacs in the lungs. If surfactant levels are low, the small air sacs in the lungs collapse resulting in poor oxygen delivery (hypoxia) to tissues. Infants deficient in surfactant therefore require treatment with high levels of oxygen to prevent damage to their vital organs. Unfortunately, premature infants are often deficient in antioxidants that are necessary to protect organs from injury caused by high concentrations of oxygen. The combination of surfactant deficiency and the presence of oxygen free radicals promote the development of chronic lung disease (bronchopulmonary dysplasia or BPD). BPD is a major cause of morbidity and mortality in premature infants. An estimated 50% of all neonatal deaths result from BPD or its complications. In the adult form of respiratory distress syndrome (ARDS), antioxidants such as *N*-acetylcysteine are recognized for their role in reducing the duration of acute lung injury ***(100,101)***. The rationale for using antioxidant liposomes to treat respiratory distress in premature infants or adults is certainly compelling and supported by the animal models detailed below. However, almost no clinical trials have been initiated.

7.2. Animal Models

Shek et al. ***(102)*** have discussed the general application of liposomes for improved drug delivery to pulmonary tissues. These authors point out that the delivery of drugs to the lung via liposomes is particularly useful because it can minimize extrapulmonary side effects and potentially result in increased drug retention time. In addition (as discussed previously), liposomes for delivery by inhalation or instillation can encapsulate enzyme

and/or chemical substances that cannot be delivered by an oral route. Smith and Anderson ***(103)*** demonstrated that intratracheally administered liposomes (with phosphatidyl choline, cholesterol, and stearylamine) have a long retention time (more than 5 d) in the mouse lung. Liposomes with entrapped Cu,Zn SOD and CAT (Cu,ZnSOD + CAT) liposomes were intratracheally instilled in rabbits and the alveolar distribution of the antioxidants measured after 4 and 24 h ***(104)***. The results indicate that Cu1nSOD + CAT liposomes could increase both SOD and CAT activities in distal lung cells, including alveolar type I, alveolar type II cells, and macrophages. More recent studies by Walther et al. ***(105)*** have shown that intratracheal administration of CuZn–CAT liposomes to premature rabbits can increase the lung SOD activity and protect against hyperoxic lung injury. Moreover, intratracheal delivery of SOD liposomes or CAT liposomes does not down-regulate mRNA synthesis of these enzymes in the premature rabbit lung ***(106)***.

Archer et al. ***(107)*** have made effective use of the isolated perfused rat lung to study the role of oxygen radicals in modulating pulmonary vascular tone. This group showed that the generation of oxygen radicals (from xanthine–xanthine oxidase) decreased pulmonary vascular presser response to alveolar hypoxia. Either pretreatment of the lung with desferrioxamine or a mixture of superoxide and CAT liposomes inhibited decreases in pulmonary vascular reactivity. SOD administered free in solution or combined with CAT in liposomes, increased the normoxic pulmonary arterial pressure and enhanced vascular reactivity to angiotensin 11 and hypoxia ***(107)***.

In a rat model, Freeman et al. ***(108)*** have shown that intravenous injection of SOD liposomes or CAT liposomes can increase (two- to fourfold) the lung-associated specific activity of these antioxidant enzymes and also provide resistance to oxygen injury. Intravenous injection of nonentrapped (i.e., free) SOD or CAT (in the absence or presence of control liposomes) neither increased the specific lung activities of these enzymes nor provided resistance to oxygen toxicity. Similarly, intratracheal administration of SOD liposomes or CAT liposomes (negatively charged and multilamellar) to rats resulted in a significant elevation of lung SOD or CAT activity as well as resistance to pulmonary oxygen toxicity ***(109)***.

Bamard et al. ***(110)*** have demonstrated that instillation of cationic SOD + CAT liposomes in a rabbit model was effective in preventing the increase in pulmonary filtration coefficient (a sensitive index of microvascular permeability) owing to free-radical-initiated lung injury. Repair of lung injury was inhibited by inhalation of elevated oxygen concentrations. This is of particular importance to the preterm human infant who may be exposed to elevated oxygen concentrations for weeks or months that could result in the chronic pneumopathy known as bronchopulmonary dysplasia. Treatment with liposome-encapsulated SOD and CAT conferred protection against the cytotoxic effects of 50 and 95% oxygen ***(111,112)*** and also protection against cell death ***(113)***.

Briscoe et al. ***(114)*** have evaluated the delivery of SOD to cultured fetal rat pulmonary epithelial cells via pH-sensitive liposomes. A fivefold increase in cellular SOD activity was observed after incubating the cultured cells with the pH-sensitive SOD liposomes ***(114)***. Fetal pulmonary epithelial cells express a high affinity receptor for surfactant protein A (SP-A). This receptor can be used to target liposome delivery to these cells by incorporating SP-A during the preparation of the SOD liposomes ***(114,115)***. The presence of SP-A in the SOD liposomes facilitates their uptake by pulmonary epithelial cells ***(114,115)***.

Considerable evidence suggests that oxidative injury to lung tissues can be mediated by neutrophils ***(116)***. Phorbol myristate acetate (PMA) has often been used to induce neutrophil-mediated lung injury in animal models, It is significant, therefore, that liposomes (dipalmitoylphosphatidylcholine) with α-TOH are able to counteract some PMA-induced lung injury in a rat model ***(116)***. In contrast, rats pretreated with blank liposomes (no α-TOH) showed no protection from PMA-induced lung injury ***(116)***.

Paraquat has also been used to induce oxidative lung injuries in animal models ***(2,10)***. Suntres and Shek ***(10)*** have compared the ability of α-

TOH liposomes or liposomes with both α-TOH and glutathione (TOH + GSH liposome) to inhibit paraquat-induced lung damage in a rat model. Lung damage was assessed by increases in lung weight (caused by edema) and decreases in lung activities of angiotensin-converting enzyme (ACE) that reflects damage to endothelial and alveolar type II epithelial cells. These investigators found that both TOH liposomes and TOH + GSH liposomes were equally effective in preventing loss of lung ACE activity but that TOH + GSH liposomes were more effective in preventing injury to alveolar type II epithelial cells ***(10)***. Interestingly, neither antioxidant liposome was effective in preventing lung edema ***(10)***.

Liposomes encapsulated with CAT have also been found to be efficacious in preventing chronic pulmonary oxygen toxicity in young rats ***(117)***. In this work, rats were treated with 100% oxygen for 8 d and also given daily intratracheal injections of the CAT liposomes (with 160 U of CAT) that prevented chronic lung toxicity. Liposomes encapsulated with SOD or with lower levels of CAT (50 or 70 U) did not prevent the chronic lung changes. SOD + CAT liposomes are also effective in protecting lung tissues from bleomycin-induced injury as evidenced by decreased levels of lipid peroxidation products ***(118)***.

Muzykantov ***(119,120)*** has pointed out that pulmonary vascular endothelial cells are not readily accessible from the airways and protecting them from oxidative stress is better accomplished through the circulatory system. To improve both the targeting and intracellular delivery to endothelium, this investigator has used a strategy in which antioxidant enzymes are first conjugated with antibodies against endothelial antigens, such as ACE, or adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) or platelet endothelial cell adhesion molecule-1 (PECAM-1). Muzykantov reports that these immunoconjugates accumulate in the pulmonary vasculature of intact animals, enter endothelium, and increase antioxidant defense capacity ***(120)***. Moreover, the ICAM-1 and PECAM-1 immunoconjugates could potentially decrease inflammatory processes in the lung by decreasing the infiltration of leukocytes ***(120)***.

7.3. Antioxidant Liposomes for the Treatment of Pulmonary Infections

As detailed above, there is an increasing body of information on the role of antioxidant liposomes in modulating pathophysiological processes in the lung. It is not surprising, therefore, that various researchers have explored the potential role of antioxidant liposomes in preventing pulmonary damage during lung infections. Suntres et al. ***(121,122)*** have used lipopolysaccharide (LPS)-induced lung injuries as a model in which to study the potential prophylactic role of antioxidant liposomes. This is a very reasonable model because Gram-negative bacteria have LPS as a component of their cell wall, and it is a potent stimulus for the generation of ROS and RNOS by phagocytic cells. LPS-induced lung injury is an excellent model for acute respiratory distress syndrome caused by sepsis. Suntres and Shek ***(121)*** found that pretreating Sprague-Dawley rats with α-TOH liposome, by intravenous administration, could significantly reduce LPS-induced lung injury. This group also found that liposome-entrapped dexamethasone was effective in preventing LPS-induced lung injury in rats ***(122)***.

8. Future Directions

Influenza is a viral disease that affects the respiratory tract. The three types of influenza viruses are designated A, B, and C, with the A and B types primarily being responsible for the yearly winter epidemics. The influenza viruses continually mutate over time causing antigenic drift, which can result in large populations of people being devoid of antibody protection. The resulting periodic pandemics can cause large numbers of deaths. For example, the 1918–1919 influenza pandemic resulted in the death of approx 20 million people worldwide. Increasing evidence suggests that free radical production and lipid peroxidation play a major role in the damage caused by influenza infection ***(123–125)***. In particular, influenza infection is accompanied by an increase in the production of superoxide radicals and decrease in the activity of

SOD that removes superoxide radicals ***(126)***. It has been suggested that the high production of ROS in the lung during influenza infection could inactivate protease inhibitors resulting in a damaging increase in protease activity ***(127)***. Administration of SOD has been found to be very effective in preventing mortality owing to influenza infection in animal models ***(128)***. It has also been suggested that antioxidants along with protease inhibitors could be useful in the treatment of severe influenza infection ***(129)***. It is surprising, therefore, that antioxidant liposomes have not yet been used for the treatment of severe influenza infection. This is certainly an area requiring further investigation considering its enormous health-related significance. Also of interest is that the influenza virus causes apoptosis in macrophages that can be prevented by the antioxidants NAC or pyrrolidine dithiocarbamate ***(130)***. Liposomes are readily phagocytized by macrophages, and hence this cell type is a natural target for antioxidant liposomes. Similarly, antioxidant liposomes are very likely to be effective in ameliorating the pathophysiology associated with severe acute respiratory syndrome (SARS), which is caused by an atypical coronavirus. SARS has an extremely high mortality rate (currently up to 15–19%) and has expanded to over 25 countries.

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