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Nanomedicine in the ROS-Mediated Pathophysiology: Applications and Clinical Advances

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Abstract

Reactive oxygen species (ROS) are important in regulating normal cell physiological functions, but when produced in excess lead to the augmented pathogenesis of various diseases. Among these ischemia reperfusion injury, Alzheimer's disease and rheumatoid arthritis, are particularly important. Since ROS can be counteracted by a variety of antioxidants, natural and synthetic antioxidants have been developed. However, due to the ubiquitous production of ROS in living systems, poor in vivo efficiency of these agents and lack of target specificity, the current clinical modalities to treat oxidative stress damage are limited. Advances in the developing field of nanomedicine have yielded nanoparticles that can prolong antioxidant activity, and target specificity of these agents. Thus, catalytic antioxidants such as recombinant superoxide dismutase (SOD), in combination with platinum and cerium oxide nanoparticles manifest higher efficacy at smaller doses with potentially lower toxicity. This article reviews recent advances in antioxidant nanoparticles and their applications to manage oxidative stress-mediated diseases.

1. Introduction

Besides their role in normal cell physiological function and cell to cell signaling, free radicals have been implicated in the pathophysiology of numerous disease processes[1,2]. Overproduction of highly reactive radical species or their precursors leads to oxidative stress, which has been observed in cardiovascular disease[3], cerebrovascular stroke[4], Alzheimer's disease[5], arthritis[6], diabetes[7] and cancer[8], among others. Considerable research has been performed in bolstering endogenous antioxidant capacity[9], administering natural antioxidants[10], and synthesizing novel antioxidants[11] to combat oxidative damage.

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Cardiovascular disease is one of the most prevalent global health conditions, and claims 17.3 million lives annually as estimated by the World Health Organization. Studies indicate that reactive oxygen species (ROS) are implicated in the pathogenesis of various aspects of cardiovascular disease, including ischemic heart disease, ischemia/reperfusion (I/R) injury, atherosclerosis, and congestive heart failure[12]. Aberrant production of oxidants due to xanthine oxidase, nitric oxide synthase (NOS) and Fe-catalyzed reactions lead to the enhanced intracellular Ca^{2+} overload, lipid peroxidation, and apoptosis of vascular cells. Similar mechanisms are known to drive the pathogenesis of acute cerebral ischemic stroke, where the oxidative damage causes cerebral swelling and degradation to the blood brain barrier (BBB). Reperfusion after an ischemic stroke, although necessary to restore cellular functions, causes high concentrations of ROS production which often manifest more harm than the initial ischemic injury. The $O_2^{\cdot -}$ generated during this process is able to react with nitric oxide (NO) produced from nitric oxide synthases (NOS)[13] to form peroxynitrite ($ONOO^-$), which is highly reactive and causes aberrant oxidation and nitration of DNA and proteins. Nitric oxide generated during I/R is helpful in promoting vasodilation, thus $O_2^{\cdot -}$ should be targeted to prevent downstream formation more oxidizing ROS, particularly $ONOO^-$.

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of modified proteins such as amyloid-β (Aβ) plaques that have an affinity for interacting with inorganic cations like Cu^{2+}, Fe^{3+}, and Zn^{2+} [14]. These reactions with macromolecules catalyze the formation of ROS via Fenton reaction to cause neurodegeneration. While the development of oxidative stress may be secondary to AD, the ROS-mediated neuronal damage plays an important role in the disease pathogenesis through progressive decline in neuron function. Rheumatoid arthritis (RA) is an autoimmune disease of the joints. Macrophages and activated T-cells attack healthy tissue partly through oxidative mechanisms via nuclear factor kappa-B (NF-κB), tumor necrosis factor-alpha (TNF-α) and nuclear factor erythroid 2-related factor (NRF2) activation, which leads to an increased inflammatory milieu and progressive joint destruction[6].

Oxygen-containing free radical molecules and their precursors formed in biological systems are collectively termed ROS, which includes superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$). Additionally, reactive nitrogen species (RNS), such as NO and $ONOO^-$, have similar dynamics and are therefore investigated to understand the pathobiology of oxidative stress related conditions. Superoxide anion is formed *in vivo* by xanthine oxidase[15], NADPH oxidase[16], activated immune cells[17], or leakage from the electron transport chain of mitochondria[18], and is regulated by enzymes such as superoxide dismutase (SOD) and peroxidases, as well as endogenous supplies of antioxidants such as glutathione (GSH). Under the condition of oxidative stress, ROS and RNS are produced in excess leading to lipid peroxidation, protein oxidation and nitration, and DNA fragmentation that ultimately affects cell membrane structures, enzyme functions, and gene expression (Figure 1).

2. Small Molecule ROS Scavengers

2.1. Edaravone (Radicut®)

Recent advances in research on the role of ROS in disease pathogenesis have propelled therapeutic strategies towards the development of ROS scavengers for clinical application. Currently, the only drug clinically approved as a free radical scavenger is Edaravone (Radicut®) for treatment of cerebral ischemic stroke in Japan since 2001[19]. Edaravone has not been clinically evaluated for its antioxidant capability, however *in vivo* studies suggest its therapeutic mechanism is through scavenging free radicals, mainly $HO^{\cdot}$ and upregulating endothelial NOS (eNOS) expression in cerebral tissue when administered with a thrombolytic agent during the onset of an acute ischemic stroke[20]. While increased eNOS activity will generate more NO, the antioxidant property of Edaravone reduces the formation of $ONOO^{-}$ [21]. Further *in vivo* studies suggest that Edaravone prevents the oxidation of low density lipoprotein (LDL), a resource to the formation of atherosclerosis, and a concomitantly scavenges lipid peroxyl radicals[22]. The combination of these effects has been instrumental in reducing neuronal damage, cerebral edema, and consequently improving patient recovery. Edaravone administration reduced the white matter lesions after hypoperfusion[23], and significantly improved the motor function 21 days after treatment in rats[24]. Edaravone is currently in clinical use only for acute ischemic stroke, although its ROS scavenging properties suggest its possible use for other diseases involving oxidative damage, such as cardiovascular diseases. The clinical use of Edaravone has been limited due to its renal toxicity among other adverse effects such as hepatic/biliary dysfunction and platelet reduction[25]. Concomitant administration with lipoic acid was shown to reduce these adverse effects while maintaining neuroprotection in a rat model of ischemic stroke[26].

2.2. N-Acetylcysteine

Thiols such as endogenous glutathione (GSH) act as cellular antioxidants by reducing ROS generation and ‘repairing’ carbon-centered radicals formed on DNA and proteins during oxidative stress by donating a hydrogen atom. N-acetylcysteine (NAC) is an antioxidant that has been used as a mucolytic agent and treatment for acetaminophen toxicity. Similar to GSH, NAC’s antioxidant property is due to its reduced thiol moiety that allows scavenging $HO^{\cdot}$ and $^{\cdot}CH_3$ radicals, with a little to no physiological activity towards $O_2.^{-}$ [27] or $ONOO^{-}$ [28]. Furthermore, NAC is a metal chelator that mediates its antioxidant effects by suppressing Fenton-type reactions of free Fe^{3+}, Cu^{2+} and Zn^{2+} ions. In human studies, intravenous NAC administered with nitroglycerin and streptokinase was shown to reduce oxidative damage from I/R injury to the left ventricle of the heart after a myocardial infarction[29]. This was attributed to $HO^{\cdot}$ scavenging and inhibition of angiotensin converting enzyme by NAC. While some studies show improvement due to the radical scavenging ability of NAC, this molecule is unable to penetrate cell membrane and blood brain barrier (BBB), which reflects as well in its low bioavailability when given orally. Under oxidative stress however, NAC was observed to cross cell membranes[30], most likely due to the increased permeability of the membranes under these pathological conditions. NAC has also been shown to be effective in the regulation of chronic ROS overproduction through inhibition of pro-inflammatory cytokines due to its conversion to glutathione. It was observed that NAC down-regulated NO-induced interleukin-1 beta (IL-1β) and tumor

necrosis factor-alpha (TNF-α) production in human chondrocytes to reduce apoptosis [7]. NAC is currently involved in clinical trials for various diseases involving oxidative stress such as Sickle Cell disease and Parkinson's disease (Clinicaltrials.gov NCT01849016 and NCT01470027, respectively). In these studies, the antioxidant capacity of NAC will be evaluated by measuring oxidative markers in blood or cerebrospinal fluid as primary or secondary outpoints.

2.3. NXY-059 (Cerovive®)

NXY-059 is an experimental α-phenyl-tert-butyl nitrone (PBN)-derived antioxidant that is capable of trapping free radicals [31], which is characteristic of the nitrone family. This results in the formation of a more stable radical molecule that is easily detected by electron paramagnetic resonance (EPR) spectroscopy, before decomposing to release NO[32]. In addition to free radical spin-trapping, NXY-059 has been shown to act as a protective agent in ischemic injury through the prevention of mitochondrial dysfunction and reduction of cytochrome *c* release that resulted in increased cell survival[33]. NXY-059 has shown great promise in pre-clinical studies, however its use in the SAINT-I and SAINT-II double-blinded placebo controlled clinical trials for the treatment of acute ischemic stroke within 6 hours of onset proved ineffective[34]. Similarly, the experimental antioxidant drugs Tirilazad[35] and Ebselen[36] were found ineffective in treating acute ischemic stroke in clinical trials, causing a major setback in the advancement of antioxidants as therapeutics.

2.4. Natural Antioxidants

Several natural antioxidants have been used in pre-clinical and clinical applications as treatments for diseases driven by free radical mediated pathophysiology. These compounds benefit from their ease of use and good bioavailability as most are common in foods or available in over-the-counter formulations. Vitamin E is a lipophilic antioxidant that has been shown to act on lipid radicals to break the chain of peroxidation in cell membranes, and is currently involved in clinical trials for AD, RA, and ischemic stroke (Clinicaltrails.gov: NCT00040378, NCT00399282, and NCT01578629, respectively). The ability of vitamin E to scavenge other radicals is limited, as evidenced by numerous negative clinical experiments (Clinicaltrials.gov: NCT00363129, NCT00117403); however its use in combination with other antioxidants like vitamin C or selenium may overcome this deficiency. Polyphenols such as resveratrol, found in red wine, and catechins such as epigallocatechin-3-gallate (EGCG) found in green tea, are emerging antioxidants that also exhibit anti-inflammatory properties as well, making them attractive candidates for the treatment of RA and other chronic inflammatory diseases [37]. EGCG has been shown to scavenge $O_2^{\cdot -}$ and $HO^{\cdot}$ radicals, as well as down-regulate pro-inflammatory cytokines and up-regulate antioxidant enzymes like SOD, catalase and glutathione peroxidase, which provides a multi-faceted approach to treat oxidative stress conditions. While natural antioxidants carry less toxicity concerns than synthetic antioxidants, they are limited by their lack of target specificity, and are found to be more effective in concert rather than treatments with a single natural antioxidant[38].

2.5. Synthetic Antioxidants

The development of synthetic antioxidants that can be 'customized' to have enhanced pharmacological activity is an active field in inflammation research. Chemical substitutions to natural antioxidants can yield products with more cell specificity, enzyme selectivity, and increased reactivity towards upstream ROS like $O_2^{\cdot -}$. Spin-quenching antioxidants or spin trapping agents are compounds that are 'stable' radicals that yield non-radical products upon reaction to ROS. Examples of spin-quenching molecules include 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)[39], a nitroxyl radical, or trityl radicals such as perchlorotriphenylmethyl (PTM-TC)[40]. Nitroxyl molecules are able to act as self-replenishing antioxidants to convert $O_2^{\cdot -}$ to H_2O_2, although they have been shown to have short *in vivo* half-lives and may induce hypotension when in circulation[41]. The nitroxyl 4-hydroxy-TEMPO (TEMPOL) has also been shown to cause cell-cycle arrest in breast cancer cells by p21 overexpression, leading to apoptosis by DNA fragmentation[42]. P21 overexpression has been shown to be detrimental in oxidative-stress conditions by suppressing glutathione peroxidase and SOD[43], thus application of nitroxyl antioxidants should take caution of this cytotoxic effect. Trityl radicals exhibit considerably high reactivity toward $O_2^{\cdot -}$ (as high as 8.3×10^8 $M^{-1}s^{-1}$ for PTM-TC)[40] while retaining some degree of inertness towards other oxido-reductant species[44]. In contrast, spin-generating synthetic antioxidants are diamagnetic molecules that form paramagnetic species upon reacting with free radicals. Hydroxylamines such as 1-hydroxy-4-phosphono-oxy-2,2,6,6-tetramethylpiperidine (PP-H) contain an N-hydroxy group that is oxidized by $O_2^{\cdot -}$ to form a more stable nitroxyl radical and H_2O_2[45]. The H_2O_2 formed can then be degraded by catalase, however H_2O_2 can form more unstable ROS, which poses a problem for the use of hydroxylamines *in vivo*. Nitrones are a family of antioxidants that are classified as linear, phenyl N-tertiary butyl nitrone (PBN)-derivatives (such as NXY-059), or cyclic, 5,5-dimethyl-1-pyrroline-N-oxide (DMPO)-derivatives (such as 5-diethoxyphosphoryl-5-methyl-1-pyrroline-N-oxide, DEPMPO). Each class is able to react with radical species to form a more stable radical adduct. These molecules are commonly used as radical probes in a technique called spin-trapping, as the radical adducts formed can be detected by EPR spectroscopy which can give spectra that are unique to each radical species formed. Nitrones are able to spin-trap multiple species of ROS, RNS[46], and carbon centered radicals, and thus makes them target specific with improved reactivity[47] and radical adduct stability[48]. In addition, nitrones also exhibit anti-inflammatory properties through down regulation of pro-inflammatory mediators. For example, PBN was shown to act on the mitogen-activated protein kinase (MAPK) cascade in human osteoarthritis chondrocytes[49], while DMPO was found to downregulate inducible NOS (iNOS) as well as inhibit the phosphorylation of MAPKs[10]. Currently, nitrones suffer from varying cellular toxicity, and fairly slow reactivity toward $O_2^{\cdot -}$[50], and the recent clinical failure of NXY-059 has hindered their application.

3. Nanomedicine ROS Scavengers

Currently, the limitations of small-molecule natural and synthetic antioxidants include low solubility, poor bioavailability, and their lack of specificity. Nanomedicine involves the utilization of molecules ranging from as small as <10 nm (e.g. metal nanocrystals)[51], to

macromolecules as large as 300 nm[52]. Polymeric nanoparticles are used to encapsulate or incorporate small molecules to provide protection from degradation or to aid in absorption and distribution of natural antioxidants. Nanoparticles can provide higher solubility to poor water-soluble compounds and enhanced surface functionalization to yield target-specificity. Inorganic metal nanoparticles are also being used for their catalytic properties, which is enhanced by their relatively large surface areas. However, toxicities have been observed as some of the nanoparticles are known to accumulate in tissues such as liver and brain[25], and others are known to have pro-oxidant properties[26]. This can be avoided by using biodegradable carrier molecules, such as albumin or poly(lactic-co-glycolic) acid (PLGA), that can be broken down by lysosomal or hydrolytic degradation of the matrix polymers[53].

3.1. Catalytic ROS-Scavenging Nanoparticles

3.1.1. SOD-containing Nanoparticles—The application of nanomedicine in ROS-mediated pathologies has dramatically advanced the strategies to the scavenging of free-radicals under oxidative stress, including target-specificity, increased cell membrane permeability, and the use of catalytic scavengers. The obvious advantage of using a catalytic ROS scavenger is that the compound is not depleted during the reaction and can potentially neutralize numerous ROS molecules, which can elevate higher potency with a lower dose. Endogenously, cells use SOD to catalyze the neutralization of $O_2^{\cdot -}$ to O_2 and H_2O_2. Nanoparticles were engineered with recombinant SOD conjugation to allow effective cellular delivery of the enzyme under oxidative stress conditions, while protecting the enzyme and avoiding its degradation in the serum[54]. The conjugation of SOD to nanoparticles also promotes BBB permeability, which allows the application of these nanoparticles to I/R injury in the brain. Upon reaching the cells, the nanoparticle is endocytosed, and the enzyme is able to catalyze the degradation of $O_2^{\cdot -}$. Reddy et al, used SOD-conjugated poly (D,L-lactic-co-glycolic acid) (PLGA) nanoparticles to treat I/R injury in the brains of rats to achieve sustained SOD delivery that enhanced the rate of survival and improved their neurological function[54]. An infusion of SOD nanoparticles during reperfusion reduced the infarct size by 65% over a saline control and 40% better than SOD in delivered in solution. Chen et al, recently engineered silica nanoparticles conjugated with recombinant Cu, Zn-SOD containing a His-tag domain for attachment to the nanoparticle, as well as a human immunodeficiency virus (HIV) transactivator protein (TAT) domain which allows enhanced transmembrane delivery[55]. Using a novel delivery approach, the authors denatured the enzyme while attached to the nanoparticle before its delivery into cells, where it was shown to be re-folded to regain its catalytic activity.

3.1.2. Platinum Nanoparticles—Platinum has been used clinically as a chemotherapeutic, as cisplatin for example, and in chemistry as a catalyst for hydrogenation and oxidation reactions. It has been shown to catalytically convert $O_2^{\cdot -}$ to H_2O_2, and H_2O_2 to H_2O and O_2, which makes it an attractive candidate as a SOD/catalase mimetic for the treatment of oxidative stress[56]. Studies have shown the efficacy for platinum nanoparticles *in vitro* conditions, where it was shown to scavenge peroxyl radicals as well[57]. However, it is not yet clear if they are able to scavenge $HO^{\cdot}$, which are the most potent ROS present under oxidative stress conditions. In a similar study, Kim et al, used HIV TAT-conjugated platinum nanoparticles in an *in vivo* model of oxidative stress to increase the uptake of the

nanoparticles into cells[58]. It was found that the TAT-conjugated platinum nanoparticles achieved similar antioxidant effects at one hundredth of the dose of non-derived platinum nanoparticles, which allows a much higher bioavailability with less degree of toxicity. The TAT-conjugated platinum nanoparticles were shown to increase survival of *C. elegans* under both acute oxidative stress, elicited by paraquat exposure, as well as chronic endogenous ROS, thus could be of potential therapeutic value in chronic inflammatory diseases.

3.1.3. Cerium Nanoparticles—Cerium nanoparticles (ceria) possess catalytic properties similar to that of platinum nanoparticles due to their ability to convert $O_2^{\cdot -}$ to O_2, to generate Ce^{3+} from Ce^{4+}, and then auto-regenerate Ce^{4+} from the reduction of Ce^{3+} or by reaction with $HO^{\cdot}$ [59,60]. Ceria were also found to catalyze the degradation of H_2O_2[61], which depicts a multi-faceted mechanism to its antioxidant properties. Furthermore, ceria were determined to decrease NO production from macrophages in mouse cells through a down-regulation of iNOS, which creates anti-inflammatory effects[62]. In another study, ceria exhibited a scavenging activity for $ONOO^-$, a potent RNS generated from the reaction of $O_2^{\cdot -}$ and NO[63]. These properties make ceria particularly useful for chronic ROS-mediated inflammatory diseases like RA, where scavenging of NO generated from iNOS in macrophages can stop further amplification of inflammatory damage. *In vitro* studies provide conflicting evidence of ceria toxicity in different cell lines, which may partly be attributed to the size and surface area of the ceria particles, with larger particles exhibiting greater toxicity[64]. Estevez et al, investigated the effects of ceria in an ischemic model of mouse hippocampal brain slices and found an approximately 50% reduction in cell death likely due to a marked decrease in the levels of ROS and $ONOO^-$ [11]. Ceria can be readily taken up by the cells, however, their tendency to form aggregates in the cytoplasm has partly limited their antioxidant properties[65]. We postulate that this could potentially be ameliorated by using a carrier polymer to inhibit the aggregation while allowing the ceria to react with ROS.

3.2. Site-Directed Nanoparticles

3.2.1. H_2O_2-Sensitive Nanoparticles—Due to the ubiquitous production of ROS under normal physiological conditions, a tissue-specific targeted approach to ROS scavenging in oxidative tissue injury can be a valuable tool in an efforts to increase antioxidant efficacy. The production of certain molecules in response to oxidative stress make them a useful target for the delivery of antioxidants. Lee et al, developed an antioxidant nanoparticle insensitive to H_2O_2, a common ROS produced during oxidative stress conditions[66]. This copolyoxalate containing vanillyl alcohol (PVAX) particle is structured with peroxalate ester linkages that degrade upon reaction with H_2O_2. This causes the release of the antioxidant vanillyl alcohol, which reduces ROS production and thus elicits anti-inflammatory effect. Vanillyl alcohol was shown to down-regulate the expression iNOS and cyclooxygenase-2 (COX-2) to reduce inflammation [67], which was markedly improved with the use of the PVAX nanoparticles. The administration of PVAX to a mouse model of I/R injury effectively suppressed oxidative stress-induced damage in both hind-limb I/R and hepatic I/R injury.

3.2.2. Mitochondria-Directed Nanoparticles—Mitochondria play a pivotal role in the production of ROS in living systems. A leakage from the electron transport chain may cause an aberrant superoxide anion production in mitochondria, condition termed as 'mitochondrial disease', which has been closely linked to diseases such as type 2 diabetes and AD[68]. Therefore, targeting mitochondria for the delivery of antioxidants can potentially regulate ROS generation from this cellular source to reduce ROS damage and improve overall cellular functions. In this regard, triphenylphosphonium (TPP) is a molecule known to readily cross cell membranes and accumulate in mitochondria of cells due to its lipophilic cation. Marrache et al, conjugated PLGA-b-poly(ethylene glycol) (PEG) nanoparticles with TPP to allow enhanced protection and improved site-directed delivery of mitochondria-targeting chemotherapeutics[69]. The researchers loaded the PLGA-b-PEG-TPP nanoparticles with curcumin, a known antioxidant of therapeutic value in AD, to deliver it more effectively to cultured human neuroblastoma cells for enhanced neuroprotection against Aβ than free curcumin. The use of mitochondrial-directed nanoparticles provides a novel approach for site-specific delivery of ROS scavengers to blunt chronic oxidative pathologies by reducing the risk-to-benefit ratio.

3.2.3. pH-Sensitive Nanoparticles—Nitroxyls such as TEMPO are stable radicals that scavenge free radicals to form two non-radical species. They also partially mimic SOD, due to their ability to self-regenerate under oxidative conditions. The *in vitro* success of nitroxyls could not be replicated in a similar fashion *in vivo* due to their hypotension inducing effect, which can be detrimental in cardiovascular or cerebrovascular I/R injury, and also due to their susceptibility to reduction by endogenous circulating molecules resulting in deactivation. Marushima et al, have developed a micelle nanoparticle with encapsulated 4-amino-TEMPO that offers *in vivo* protection to the nitroxyl molecules[70]. The study showed that the radical containing nanoparticle (RNP) significantly decreased infarct size in a rat model of acute cerebrovascular I/R injury over saline control and free TEMPOL molecules. Interestingly, there was no observed effect on blood pressure by the RNP molecules in contrast to the effect of free TEMPOL that produced a drop in blood pressure. In addition, the RNP molecules showed an in vivo half-life 60-times longer than TEMPOL.

At the site of ischemia, a large reduction in tissue pH occurs, which returns to the normal levels upon reperfusion, a phenomenon commonly referred to as the 'paradox of reperfusion injury'[71]. Recent exciting development in the field of nanomedicine has yielded pH-sensitive nanoparticles capable of releasing their contents in response to a drop in pH levels. The micelle RNPs used are sensitive to pH, and are reported to form polymers under mildly acidic conditions to allow leakage of the TEMPO molecules to the ischemic area. This approach was used to design a pH-sensitive 'redox polymer' that is persistent enough to be delivered to the brain after oral administration for the treatment of chronic neurodegenerative disease[72]. The RNP micelle is broken apart in the low pH of the stomach, and polymer molecules with covalently-linked TEMPOL are absorbed. Alternatively, the pH-sensitivity of the polymer was removed to prohibit disruption of the micelle, and to promote accumulation in the colon for the treatment of colitis, a chronic inflammatory condition of the colon[73]. Overall, the protection and target specificity offered by the micelle

encapsulation of the RNPs show great promise for their application as antioxidant therapies for oxidative-stress mediated disease.

Similar to the paradox of reperfusion injury, a drop in pH occurs at the site of bacterial infection[74]. This characteristic was therapeutically exploited by Radovic-Moreno et al, to develop a nanoparticle capable of 'charge-switching' at the site of infections due to a change in pH from 7.4 to 6.0[75]. Structurally, the shell of PLGA nanoparticle was conjugated with poly-L-histidine to form a co-polymer, which was subsequently loaded with an antibiotic to be released at the target site. The nanoparticles were found to selectively bind to gram-negative bacteria due to the positive charge of the bacterial cell wall caused by the low pH, thus allowing the release of antibiotic into the bacterial cell. Using a pH-sensitive approach could facilitate higher accumulation of antioxidants in the cells during reperfusion, a period of significant ROS generation, as opposed to the post-reperfusion period in this process.

4.1. Future Directions / Conclusions

The summarized multi-faceted approaches to the delivery of antioxidants suggest the potential application of nanomedicine-based therapies hold significant advancement over conventional therapies. Due to the ubiquitous production of ROS in living systems, protection of antioxidants and their targeted delivery to the site of oxidative stress can greatly enhance their efficacy. For instance, the possibility of conjugating to the surfaces of nanoparticles to allow sensitivity to the local environment, and thus target specificity, as well as protection of their cargo while in circulation provide great promise and incremental benefit in applying such antioxidant therapies. Catalytic antioxidants like ceria, platinum, and recombinant SOD, as well as partially catalytic antioxidants like nitroxyls may loaded into nanoparticles to afford protection until they reach sites of oxidative stress. Additionally, advances in nanoparticle surface functionalization such as pH- and H_2O_2-sensitive polymers, organelle-directed molecules, and HIV TAT-conjugation may yield increased target-specificity for their antioxidant contents, a quality that is not seen in endogenous, natural or current clinical antioxidants. Taken together, combinations of antioxidant and surface functionalization can be tailored for specific disease processes and enhanced therapeutic gain. Some suggested antioxidants and recent nanoparticle advances as applied to ROS-mediated pathologies are listed in Table 1. For example, the catalytic ceria, platinum and SOD scavengers may be applied to cardiovascular and cerebrovascular I/R injury to down-regulate acute oxidative stress, while TEMPO and nitrones may provide radical scavenging capabilities along with NO donating properties. These antioxidants may be loaded onto pH- or H_2O_2-sensitive nanoparticles or mitochondrial-directed nanoparticles to yield greater target specificity, and PLGA coating can ensure BBB permeability. Rheumatoid arthritis and Osteoarthritis are characterized by chronic oxidative and nitrosative stress as well as inflammation, which may potentially be ameliorated by EGCG or nitrones for their scavenging and anti-inflammatory properties, or ceria for their $ONOO^-$ diminishing capabilities. Similarly, AD is characterized by chronic oxidative stress, but presents the obstacle of BBB permeability. Encapsulation by PLGA with H_2O_2-sensitive conjugation can provide permeability and specificity for the antioxidants, while NAC as free radical scavenger can chelate metal ions to prevent Fenton reactions. However, rigorous research is warranted in this area to further validate these promising therapeutic approaches

that capitalize the nanotechnology platform for better treatment options and newer generation of drugs.

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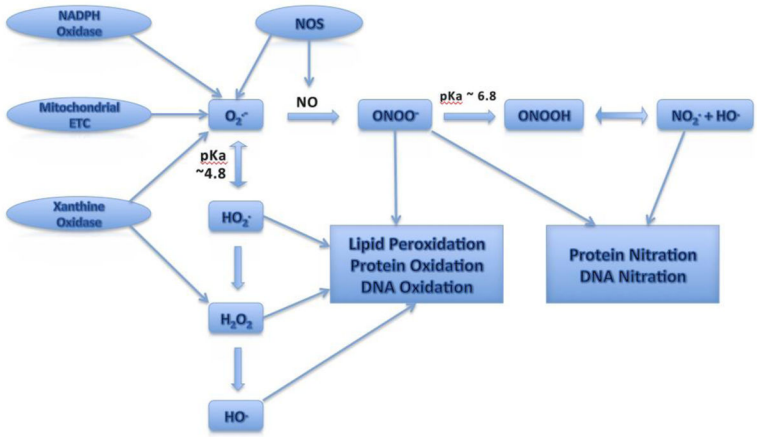

Figure 1.
Schematic diagram of ROS and RNS generation during oxidative stress

Table 1

Selected ROS-Mediated Pathologies with Respective Applicable Antioxidants and Nanomedicine Advances

Pathology	Antioxidant / Scavenger	NP / Conjugation
Cardiovascular/ Cerebrovascular I/R • Acute oxidative stress • Lowered pH	SOD Platinum NPs Ceria TEMPO Nitrone	PLGA TPP Poly-L-His / Micelle Peroxylate ester
RA / Osteoarthritis • Chronic oxidative / nitrosative stress • Inflammation	EGCG Vanillyl Alcohol Ceria Nitrone	PLGA TAT
AD • Chronic oxidative stress • BBB • Inorganic cations	NAC TEMPO Nitrone	PLGA Peroxylate ester