Effects of ROS and Aging on Cellular and Organ Function

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In aged individuals, increased oxidative damage in cells leads to cellular decline and organ dysfunction. Since the mid-1950s, reactive oxygen species (ROS) have been implicated in age-related organ damage, including hepatic, cardiovascular, and renal disease. While this connection has been established for some time, the production and particularly the function of ROS within and between cells is still being studied. This review will examine what is known about the enzymatic and mitochondrial sources of ROS within cells, and explore their effect on age-related diseases in the liver, heart, and kidneys. Fibrosis and functional decline of each of these organs has been reported in animal models and clinical data. ROS including hydroxyl radicals (OH$^{\cdot}$), hydrogen peroxide (H_2O_2), and superoxide anion (O_2^{-}) are discussed as well as processes that produce them: P450 enzymes, xanthine oxidase, NADP oxidase, and the electron transport chain (ETC). While ROS are useful for cell signaling, accumulation of these species at high levels is damaging. This review will focus on the role of ROS on cell-signaling proteins including NF-κβ, TNF-α, and atrial natriuretic factor (ANF), as well as resulting mitochondrial DNA (mtDNA) damage. The modulation of these factors by high ROS prevalence, in addition to a decline in mtDNA damage repair capabilities over time, is the foundation of age-related oxidative stress. As such, implications of reductive enzymes including superoxide dismutase, catalase, and glutathione peroxidase are discussed as well.

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1.0 Introduction to Oxidative Stress

ROS exist in cells as mediators of inflammation and indicators of damage. Even in young, healthy cells, ROS are produced constitutively through mitochondrial ATP production and cell metabolism. If oxidative species are present in high concentrations, however, cellular damage occurs. In 1956, Harman proposed that lifelong exposure to oxidative damage within the body is largely responsible for the aging process [1]. Over time, oxidative species accumulate in nearly every tissue, and reduction processes of the body cannot keep up. ROS are problematic in nature due to the presence of oxygen atoms with unpaired electrons. Species that commonly accumulate in tissues include hydroxyl radicals ($OH^{\bullet}$), superoxide anion (O_2^-), and hydrogen peroxide (H_2O_2) [2]. Scenes of ROS production include the mitochondria, hepatic metabolism, and vascular NADH/NADPH oxidase. The macromolecules that become affected by this production include carbohydrates, lipids, and DNA. Specifically, mitochondrial DNA is compromised through base modulation as well as single and double stranded breaks. In clinical practice, oxidatively modified forms of these macromolecules are used as biomarkers for quantifying oxidative stress [3]. This ROS production is offset by the enzymatic reduction of these species by molecules called antioxidants [4]. It is theorized that the dysregulation of this antioxidant-ROS balance is severely compromised by aging [1].

1.1 Senescence-Associated Secretory Phenotype

In aged cells, a halting of cellular replication can occur known as senescence. During this period, cells exhibit a senescence-associated secretory phenotype (SASP) which, with increased ROS production, contributes to cellular damage. Repeated replication of DNA and other cellular components leads to mutation and an inclination toward tumorigenesis. The role of this senescence is to halt replication in damaged cells to prevent tumor formation [5]. The SASP includes the activation of tumor suppressor genes p16 and p53, which upregulate NF-κβ and consequently TNF-α expression. Cells exhibiting the SASP are more common with advanced age, and these factors become increasingly present in the milieu [6]. In healthy cells, this senescence allows tumorigenic or otherwise problematic cells to cease reproduction, protecting organ function. Significant levels of ROS are capable of damaging cellular components which, especially in aged individuals, leads toward the SASP in a large number of cells. This creates a surplus of senescent cells that are releasing transcription factors and cytokines promoting inflammation, degeneration, and fibrosis.

1.2 Production of ROS

Over the course of the human lifetime, we are exposed to ROS both endogenously and exogenously. Exogenous sources include cigarette smoke, pollution, and ionizing radiation. These sources of ROS, though important causes of disease, are not as prominent in aging as endogenous sources, and will not be discussed here.
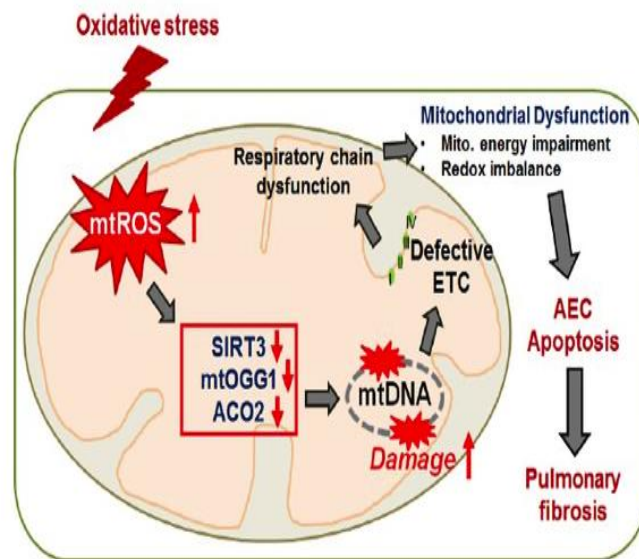
Figure 1. Model of mtDNA damage caused by oxidative stress. Increased mitochondrial ROS (mtROS) leads to decreased ETC functionality and increased proton leak [2].

One of the most ubiquitous endogenous ROS is the superoxide radical. The organelle mainly implicated in endogenous ROS production is the mitochondria [7]. In the mitochondrial ETC, oxygen is reduced to water using cytochrome oxidase (Complex IV) as an intermediate reservoir. During the reduction process, electrons from other complexes can leak to oxygen atoms

which become superoxide radicals. These radicals are increasingly formed after mtDNA becomes damaged through long-term exposure to ROS. This process constitutes the basis of the mitochondrial free radical theory of aging. ROS are capable of mutating mtDNA, which has less efficient proofreading and repair mechanisms than nuclear DNA [8]. The ETC can produce superoxide anion through complexes I and III as electrons are being shuttled through to complex IV. Additionally, the electron shuttle Cytochrome C is capable of becoming damaged due to ROS exposure. Cytochrome C damage can result in the uncoupling of this electron carrier from the ETC. This allows free electrons to react with nearby macromolecules, like mtDNA [9]. Exposure and subsequent mutation by ROS further increases the probability of electron and ROS leak, as shown in Figure 1 [10]. f in combination with the close proximity of the ETC to mtDNA, makes the mitochondria vulnerable to free radical damage. A source of ROS exists in the use of enzymes in tissues as well, including xanthine oxidase, NADPH oxidase, and the cytochrome P450 family of enzymes. Xanthine oxidase, an enzyme responsible for purine catabolism, catalyzes the breakdown of hypoxanthine into uric acid. Purine metabolism is necessary as cells are recycled, or heavy purine meals are digested like seafood, alcohol, or meats. During hypoxanthine metabolism, this enzyme produces superoxide radicals which must then be reduced by superoxide dismutase [11]. Superoxide radicals are produced by NADPH oxidase as well. NADPH oxidase is responsible for innate immunity in fighting oxygen-labile pathogenic organisms [12]. Upon recognition of a bacterial invader, NADPH oxidase is capable of producing superoxide anion that can cause damage to bacterial macromolecules. The other function of NADPH oxidase is in cell-cell communication. Release of small amounts of ROS in response to hormones like angiotensin allows one cell to communicate environmental cues to its neighbors [13]. ROS production by the p450 enzymes occurs through a variety of mechanisms. An increase in ROS production by these

enzymes can be caused by the metabolism of xenobiotics like azithromycin, which will further contribute to the ROS pool [14]. These biologic processes: the ETC, xanthine oxidase, NADPH oxidase, and P450 are all necessary components of a functioning human organism. Their ROS byproducts, however, have consequences over time, causing age-related degeneration of tissues and organ systems.

2.0 Aging and ROS

Humans, like all animals, have a finite life cycle. The start of a functional decline toward senescence can be caused by many things; one of which is long term oxidative stress (Figure 2). Species with longer lifespans than humans have both greater antioxidant activity and more efficient antioxidant systems when compared to human cells [15]. We know, therefore, that oxidative species play a part in cellular decline, but how exactly does this happen? As mentioned previously, ROS are being produced at almost all times in all cells; man requires molecular oxygen to survive. Protons that leak from the ETC are capable of reacting with molecular oxygen used in the mitochondria. This combination generates hydroxide radicals which are then further metabolized
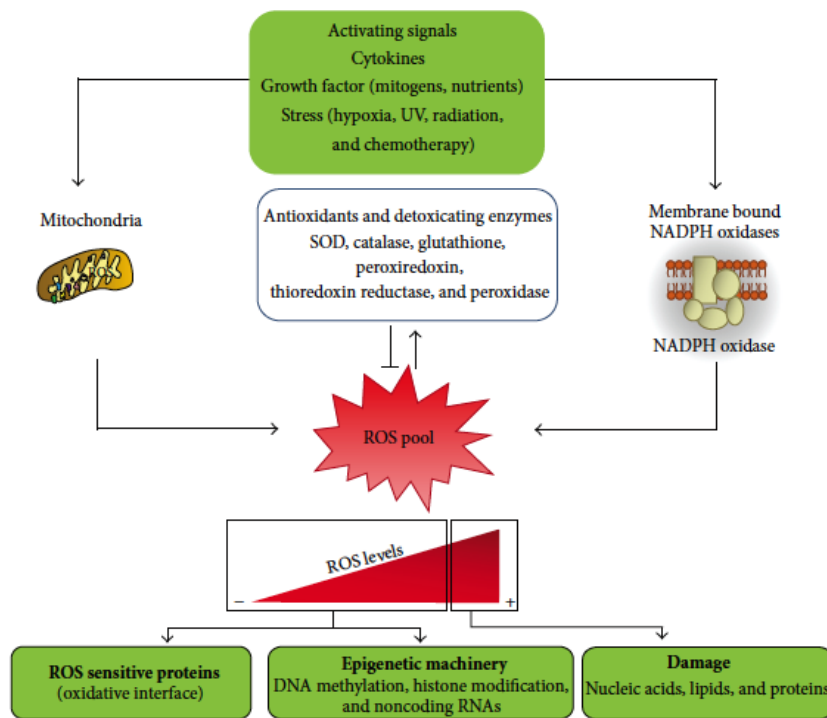
Figure 2. Summary of the ROS damage process. An increased ROS pool leads to cellular damage, while lower ROS levels affect cell messengers and machinery [3].

to create hydrogen peroxide. In cells, oxygen and water are metabolized into hydrogen peroxide via superoxide dismutase. From there, catalase and glutathione peroxidase eliminate hydrogen peroxide with water and oxygen as products, acting as antioxidants. Issues related to oxidative stress arise when a surplus of oxidants exists compared to antioxidant levels [16]. These issues are often what cause problematic age-related ailments such as liver disease, heart disease, and kidney dysfunction.

3.0 Oxidative Organ Damage

In the liver, hepatocytes are a major site of metabolism due to extensive expression of the cytochrome P450 enzymes, and high perfusion. Here, P450-mediated-metabolism of various compounds at the endoplasmic reticulum can produce high levels of reactive H_2O_2 [17]. These heme-containing enzymes are capable of binding and releasing oxygen in the absence of their substrate. This release of oxygen has the opportunity to interact with hydrogen and create hydrogen peroxide [18]. In an aged liver, the concentration of the catalase and glutathione peroxidase enzymes, which break down H_2O_2, become decreased due to mtDNA damage from life-long ROS exposure. This leads to less efficient clearing of the strongly oxidative peroxide molecules from hepatocytes [19]. The sustained increase in ROS, and mtDNA damage, can result in the activation of transcription factors such as Early Growth Response Protein 1 (Egr-1), Nuclear Factor Kappa β (NF-κβ), and Activator Protein 1 (AP-1), which ultimately trigger hepatic senescence and fibrosis. Dysregulation of NF-κβ is responsible for initiating the trans-differentiation of hepatic stellate cells into hepatic myofibroblasts. These hepatic myofibroblasts are responsible for extracellular matrix production, and their increased presence results in an increase in liver fibrosis [20]. NF-κβ upregulation has also been correlated with an increase in the apoptotic cytokine tumor necrosis factor alpha (TNF-α) [21]. NF-κβ directly promotes expression of NADPH oxidase, which is designed to generate superoxide as a defense mechanism against pathogens and for cell signaling. Overexpression of this enzyme, however, causes dangerous levels of superoxide to accumulate, resulting in TNF-α excretion and cell senescence [22]. This increased senescence results in fibrosis of the liver and a decrease in catabolic enzyme efficiency, which hinders the ability of aged livers to process drugs and endogenous compounds. Decreased cell count and enzyme efficiency leads

to lower organ function which can cause waste products to accumulate in aged livers, leading to failure and further dysfunction [17]. Clinically, this effect is apparent through hepatic volume and blood flow in aged individuals, which are both reduced. In 2002, a study using medical documentation found that, because of this reduction, metabolic activity was blunted enough to warrant recommendation of a starting dose of hepatically cleared drugs that is 30-40% smaller than average doses given to patients under the age of 65 [23].

Similarly, heart disease can arise from age-related oxidative stress. Superoxide anion and H_2O_2 are mediators of vascular smooth muscle cell growth and apoptosis. Increased levels of these ROS, similar to what is seen in hepatocytes, can act as a signal to induce the SASP in these cells [2]. In cardiomyocytes, high levels of mitochondria are present, which reliably produce ATP to ensure the proper function of this crucial organ. In aged individuals, increases occur in superoxide and H_2O_2 concentrations. In the aged heart, mitochondria produce significantly higher levels of ROS, which is able to damage mitochondrial DNA. This damage begins a cycle of increased mitochondrial ROS production [24]. Increased production leads to the upregulation of transcription factors including NF-kβ, resulting in senescence and apoptosis of cardiac myocytes through TNF-α. In the heart, this results in reduced cardiac volume, ventricular hypertrophy, and fibrosis, putting more strain on remaining cardiomyocytes to keep a steady cardiac output [25]. With advanced age, this lack of robust activity may lead to decreased tissue perfusion and widespread cell death. To study cardiac health, rodent models have been employed extensively and have shown a high level of biological similarity to human cardiac tissue. Commonly, aged hearts experience hypertrophy due to hypertension, but NF-κβ has been implicated in a rat model of ischemic heart disease. While this necrosis factor is responsible for TNF-α upregulation, it has also been shown to upregulate atrial natriuretic factor (ANF). ANF is largely responsible for
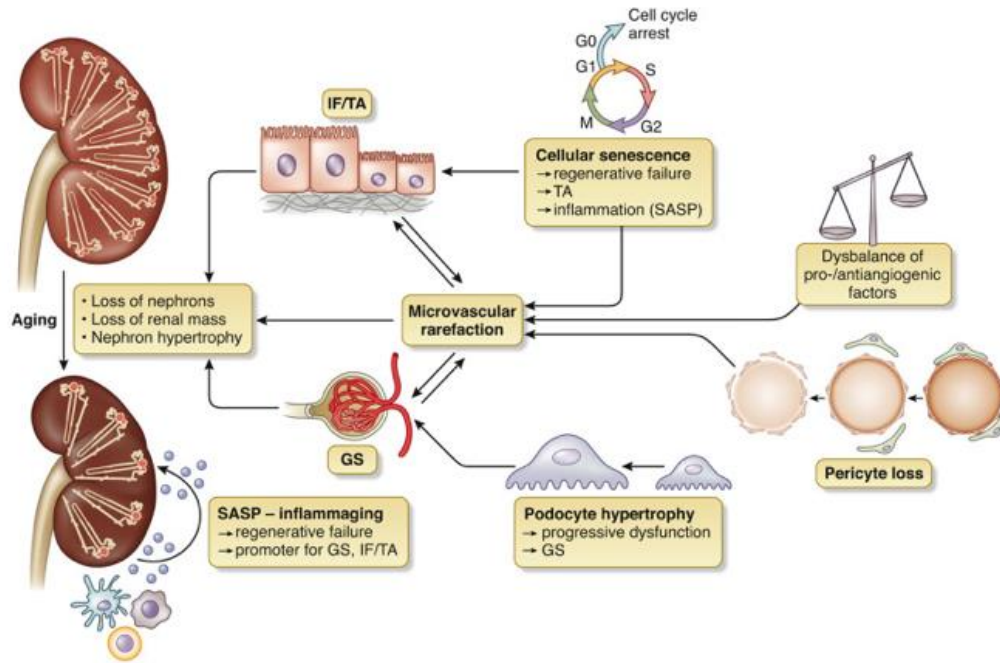
Figure 3. Age-related apoptosis of renal cells causes interstitial fibrosis, tubular atrophy (IF/TA), and glomerulosclerosis (GS). Cellular senescence due to oxidative stress reduces the regenerative capacity and function of renal filtration [1].

hypertrophic induction due to hypertension, but ROS induced NF-κβ production is positively correlated with the upregulation of ANF. As a result, reduced cardiac volume, ventricular hypertrophy, and fibrosis are directly correlated to mtDNA damage due to ROS exposure over time [26].

In the renal system, release of ROS from aged mitochondria can lead to fibrosis and kidney dysfunction. ROS release induces production of cytokines including p53 and p16, which regulate cell cycle and apoptotic activity. In a 2014 study, high levels of p16 were found in patients with age-related renal fibrosis due to mtDNA damage [27]. As is the case in other tissues, this DNA damage causes more ROS to leak from the mitochondria's ETC, causing cellular damage and apoptosis (Figure 3). Here, NF-κβ is shown to play another important role in this aging tissue. A study using a p16 deletion model in mice recently clarified the relationship between this cytokine

and NF-κβ. In p16 knockout mice, age-related renal dysfunction (increased glomerular filtration pressure and fibrosis) was improved via NF-κβ signal reduction [28]. This not only increases our understanding of oxidative kidney dysfunction, but also increases understanding of the role of NF-κβ in relation to cytokines in other tissues. Importantly, renal and cardiac oxidation are intertwined. Cardiac hypertension and hypertrophy are responsible for a decrease in renal perfusion, and therefore decreased glomerular filtration rate [29]. Together, oxidative damage to these organs can be quite dangerous and even fatal to an aged individual.

3.1.1 Current Findings in Damage Repair

In the liver, heart, and kidney, mtDNA damage is both responsible for and increased by ROS generation and decreased enzyme efficiency. Keeping in mind that catalase and glutathione peroxidase act as a natural defense to H_2O_2 [30], and that superoxide dismutase works to enzymatically reduce superoxide anion [11, 12], it is clear that external ROS mediation is necessary in the event of antioxidant failure. Non-enzymatic scavengers of ROS do exist and include the antioxidants β-carotene, vitamin C, and vitamin E. Over time, however, mitochondrial ROS causes protein damage, leading to deficiencies in the processing power of even these antioxidants [31].

Instead, to combat cell damage, xanthine oxidase is becoming a popular druggable target. Allopurinol, a drug used in the treatment of gout to reduce uric acid, acts as an inhibitor of xanthine oxidase. In rats, this drug was shown to protect the heart against myocardial infarction caused by hypertrophy and fibrosis. Inhibition of xanthine oxidase decreased superoxide and peroxide presence in myocardial tissue, with decreased rates of myocardial infarction [32]. More recently, a xanthine oxidase inhibitor has been shown to have a lower IC_{50} than allopurinol using *in vivo*

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and *in vitro* studies. This inhibitor, nitro-oleic acid, is a nitrated fatty acid and an irreversible inhibitor of xanthine oxidase. Purified xanthine oxidase exposed to either allopurinol or nitro-oleic acid showed inhibition of superoxide formation with IC_{50} of 2.25µM and 0.75µM, respectively [33].

A relationship also exists between Polynucleotide Phosphorylase (PNPase) inhibitors and oxidative stress. PNPase inhibitors block the catalytic activity of xanthine oxidase, preventing superoxide and uric acid formation. One potent inhibitor, 8-aminoguanine (8AG), has shown promise in relieving oxidative stress in the urinary system of rats. By inhibiting xanthine oxidase, 8AG disrupts metabolism of inosine to hypoxanthine and guanosine to guanine. Both inosine and guanosine display anti-inflammatory properties in tissues, especially in the lower urinary tract (LUT). Exposure of the LUT to ROS over time is correlated with urinary incontinence in humans, which may be able to be modulated through the dosing of 8AG [34].

An important facet of the discussion of hepatic and renal clearance systems is polypharmacy. Polypharmacy may play a role in the extent of oxidative damage, especially in the geriatric population. A 2007 study showed that adult patients over the age of 65 are taking about 9 medications on average [35]. Almost 50% of geriatric patients are currently taking one or more prescribed drugs that are not necessary [36]. Geriatric polypharmacy with concomitant oxidative damage to clearance organs increases serum residence time of medications, placing constant oxidative stress on organs. As mentioned previously, increased ROS presence results in decreased enzyme efficiency in hepatocytes, which causes metabolic waste products to accumulate [17].Careful prescription of medication to elderly patients, as well as patient and physician education are crucially important in reducing the aggravation of an already out of balance redox system.

4.0 Conclusion

During the aging process, our cells begin to accumulate highly potent oxidative species known as ROS. These species (peroxide, superoxide) are commonly produced by aged mitochondria containing damaged mitochondrial DNA. These organelles are capable of leaking ROS from their ETC causing damage to antioxidant enzymes and the dysregulation of senescence. Additionally, enzymes including xanthine oxidase, NADPH oxidase, and P450 produce ROS during necessary metabolism and ATP production, further contributing to the ROS pool in cells. Apoptosis markers including NF-κβ and TNF-α are upregulated upon an increased presence of ROS, causing increased cell senescence and cell cycle dysregulation in organs such as the liver, heart, and kidneys. These organs are often implicated in age-related disease and death, due to ROS exposure, which can further increase ROS production and SASP likelihood. Endogenous enzymes respond to ROS, but can have reduced function in aged individuals. To combat these challenges, compounds such as 8-aminoguanine and nitro-oleic acid are being studied to reduce oxidative damage on organs. It is worth noting that polypharmacy plays a role in increasing oxidative exposure to the elderly. Through careful prescription, patient education, and research surrounding age-related oxidative stress, improvements have and continue to be made in human longevity and ROS mediation.

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