Chapter 1
Introduction: Free Radicals in ENT Pathology

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1.1 Introduction

Basic science research has led to new understanding of the mechanisms of pathology underlying dysfunction in the ear, nose, and throat. This research is defining new interventions that modulate biochemical events leading to pathology and may one day offer new opportunities to prevent and treat impairment. Clearly, many of the advances in our basic understanding and promises for new interventions to prevent and treat pathology are based upon molecular and biochemical studies defining the primary and secondary signaling molecules controlling cellular development and homeostasis, including response to age and stress factors. A key influence increasingly appreciated as contributing to pathology, consequent to many etiological factors, and affecting essentially all tissues and organ systems is the delicate balance that must be maintained in the level of cellular free radicals. While free radicals are essential in the maintenance of normal cellular function, their excess induces cell injury and death. Free radicals directly facilitate biochemical reactions necessary for cell life and participate in cell signaling or "redox signaling,"

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e.g., nitric oxide control of vascular tone. As will be demonstrated in this book, free radical biology is emerging as a defining force determining normal function and, in excess, a common pathway to cell death associated with many etiologies leading to ENT pathology. This book will examine the current state of free radial biology as it impacts on hearing, otology, laryngology, rhinology, and head and neck function. Our intent is to highlight the interrelationship of basic and translational studies in each area, to define the challenges to translation, and to identify the existing basic issues that demand investigation as well as the opportunities for novel intervention to prevent and treat ENT pathology and impairment. In each chapter, or in some cases pairs of chapters, the authors have been charged to include and where possible marry issues of basic research with translational challenges and research, defining the pathway by which new basic insights may lead to interventions to prevent or treat impairment.

Free radicals are highly reactive species (atoms, molecules, and ions) with one or more unpaired electrons in their outer electron shell. While free radicals may be induced by a number of factors and mechanisms, in vivo free radicals are primarily a by-product of the mitochondrial respiratory chain, i.e., normal metabolism of oxygen. The primary role of the mitochondrion or "power plant" of the cell is the phosphorylation of ADP to produce ATP. The process is driven by cellular respiration (oxidization) of succinate (and /or pyruvate) and NADH, products of the citric acid cycle. The process releases electrons which then are passed through a sequential chain of four increasingly electronegative donors-acceptors to oxygen. This process provides the energy to "pump" protons through the inner mitochondrial membrane, creating a proton gradient; and the flow of protons back through that membrane, the ATP synthase complex, drives the synthesis of inorganic phosphate + ADP to ATP.

In a normal, healthy cell, a small percentage of electrons escape the electron transport chain and "leak" directly to oxygen forming reactive oxygen species (ROS), e.g., superoxide anions, hydrogen peroxide, and hydroxyl radicals. And in these normal cells, there are endogenous "antioxidant" enzyme systems to control the excess of free radicals, e.g., thioredoxin, glutathione, superoxide dismutase (SOD), catalase, and melatonin. In addition to endogenous control of antioxidants, a number of exogenous factors, typically consumed as part of our diet, contribute to the control of the number and variety of free radicals available intracellularly by directly scavenging them, e.g., carotenes, ascorbic acid, and α-tocopherol, or by modulating the efficacy of endogenous antioxidant systems, e.g., catalase, glutathione, and SOD. Antioxidants are either hydrophilic, neutralizing radicals in the water compartments of the cell (cytosol), or hydrophobic, reducing radicals in the lipid (membrane) compartments of the cell, and some of these antioxidants function extracellularly. The different sites of action and different mechanisms of antioxidant action contribute significantly to research aimed at defining potential synergistic effects of antioxidants in the control of free radicals.

In normal, healthy cells functioning within a normal operating range of energy demand, the "leak" of electrons that results in the formation of ROS is small, and the free radicals formed can be appropriately controlled by these endogenous

antioxidants.[1] However, in the cell with compromised antioxidant systems, in the cell with age-related injury and DNA damage, or in the cell under extreme environmental stress, the formation of free radicals may exceed the capabilities of the antioxidant systems available and disrupt the delicate balance required for normal homeostasis, resulting in oxidative stress, excessive free radicals, and subsequent cell injury. ROS, specifically superoxide radicals, under enzymatic control, combine with nitric oxide to form reactive nitrogen species (RNS). Excess ROS and RNS directly damage cells and tissues by destruction of cellular and intracellular membranes by lipid peroxidation and by upregulating genes controlling apoptotic pathways and mutagenic damage of DNA.

Etiological factors that can contribute to excess free radical formation affecting cell pathology, including the tissues and systems of ENT, include tobacco smoke,[2] hyper- and hypoxia, UV radiation, intense noise exposure, aminoglycoside antibiotics, chemotherapeutic drugs (e.g., cisplatin), tissue trauma (e.g., implant surgery, with cell death and/or bleeding that lead to superoxides and hydroxides, respectively), age, vascular spasm and reperfusion, viruses and bacteria (leading to cell death and tissue inflammation and secondary to immune responses), inadequate diet (leading to reduced endogenous and exogenous antioxidants), and cardiovascular disease and diabetes (see Chap. 6).

The mechanisms underlying ROS-/RNS-induced pathology are increasingly clear and clearly share some communality across etiology, free radical entity, tissue, and the molecular pathways to cell death. For example, superoxide radicals can interact with nitric oxide to produce a highly toxic molecule, peroxynitrite, that can cause nitration of critical proteins which can lead to cell death in various tissues in the head and neck. This can result in significant morbidity and loss of function in the ear, nose, and throat.

Our initial intent for this book was to provide a comprehensive summary of the work on oxidative stress as a common pathway to pathology across the fields of ENT. The disproportionate research on biology of free radicals focused on the ear and hearing made that impossible. Hence, the majority of the chapters (20 of 24) concern the ear. Three initial basic chapters on the basic biology of free radicals provide the fundamental background to appreciate the more nuanced discussion of ROS/RNS in specific pathologies of the ear that follow: the first provides a broad overview of the basic concept of free radical biology, while the following chapters in this section discuss their role in normal cell function and the increasing evidence for antioxidant interventions to prevent oxidative stress-induced pathology. The second part of the book provides a two-chapter overview (Chaps. 5 and 6) of hearing loss from an epidemiological perspective, assessing the contribution of stress factors related to aging and health to free radical pathology, epidemiological data on hearing loss from environmental factors of noise and heavy metals, and the role of

[1] Although the slow accumulation of free radicals in "normal" cells and tissues is thought to be a significant factor in normal aging and cell death, for individual cells, tissues, and the organism.

[2] As pointed out in Chap. 24, "each puff of a cigarette contains 100 trillion free radicals of varying types."

nutrition on hearing loss. Following sections assess oxidative stress in noise-induced hearing loss, drug-induced hearing loss, age-related hearing loss, and the increasing evidence for a role of free radicals in hereditary hearing loss and antioxidant intervention to prevent and treat such hearing losses. Important concepts within these areas include clinical metrics for protections. There has been little consensus in the field regarding animal models (chinchilla, rat, guinea pig, or mouse), common noise insults (which ranges from 105 to 120 dB SPL across studies), temporary threshold shift (TTS)-inducing noise or permanent threshold shift (PTS)-inducing noise, and whether it is better to measure protection against a small PTS or a large PTS. Other questions may be what day to initiate therapy relative to noise, how long to continue the therapy, and when to assess the final metrics. There has also been little systematic dose-response data collection. We do not know if we are comparing the best (most effective) dose of different drugs or simply two randomly selected doses that happened to yield benefits and were adopted for subsequent studies. These are major issues in the animal literature and are likely to plague human testing as well given the variety of metrics that are emerging for use in studies on prevention of noise-induced hearing loss (NIHL) (Chap. 9) and drug-induced hearing loss (DIHL) (Chap. 12), including conventional air conduction thresholds, extended high-frequency threshold testing, distortion product otoacoustic emissions (DPOAE) tests, auditory brainstem response (ABR) tests, and speech in noise tests. Finally, we include a part on the potential role of surgical trauma on hearing loss in animals and humans and the growing recognition that oxidative stress may play a role in other causes of hearing loss, e.g., Meniere's disease. The final part of the book provides critical chapters describing the role of free radicals on head and neck pathology, primarily cancer related, and their role in sleep apnea and in nasal and paranasal disease.

The final chapter of this book reflects a meeting of all the contributors, culminating in a discussion and "white paper" identifying the challenges to the field and where possible defining the studies and collaborations that may lead to improved understanding of free radical biology in ENT and new interventions to medically treat ENT pathology.