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Forum Editorial

Targeting Oxidative Stress for Neuroprotection

Daniel A. Linseman

The Central Role of Oxidative Stress in Neurodegeneration

The generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) leads to oxidative or nitrosative damage or both to cellular proteins, lipids, and DNA (a process collectively referred to here as *oxidative stress*). During aging, oxidative stress increases because of an aberrant generation of ROS/RNS and a gradual decline in cellular antioxidant defense mechanisms. Consequently, aging and the associated increase in oxidative stress are major risk factors for many neurodegenerative diseases. In addition, various genetic mutations and environmental exposures can sensitize individuals to oxidative stress and neurodegeneration. Within the cell, mitochondria are a major source of oxidative stress. However, additional intracellular sources of ROS and RNS exist, as well as extracellular sources such as those resulting from inflammation or exposure to toxins. A significant body of literature indicates that ROS or RNS or both resulting from mitochondrial dysfunction, neuroinflammation, or toxicants are major factors in the oxidative stress–dependent neuronal death that underlies various neurodegenerative disorders, including Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and many others (1, 8, 9, 16, 18). Accordingly, the discovery of novel strategies to mitigate oxidative stress is a principal focus of current therapeutic development programs for neurodegenerative diseases.

Cellular Targets of Oxidative Stress

If left unopposed, oxidative stress results in the accumulation of dysfunctional proteins, lipid peroxidation products, and damaged nuclear or mitochondrial DNA. Oxidative damage to proteins is a major mechanism underlying neurodegeneration, which occurs through a variety of pathways including carbonylation, oxidation of critical sulfhydryl groups, nitrosylation, and nitrotyrosination. For example, PD cybrid cells show enhanced protein carbonylation and sulfhydryl oxidation because of deficits in mitochondrial complex I activity and consequent generation of ROS (5). In particular, these types of oxidative damage are major contributing factors to the dysfunctional aggregation of α-synuclein, a principal hallmark of PD. Nitrosative damage to proteins also is capable of causing protein dysfunction. Several examples include *S*-nitrosylation of peroxiredoxin 2, protein-disulfide isomerase, and heat-shock protein 90 (12). The nitrosylation of each of these proteins results in a loss of their respective protective functions and contributes to further oxidative stress and aberrant protein misfolding and aggregation in diseases such as PD and AD.

In addition to unique proteins becoming dysfunctional or aggregated as a result of oxidation, damage to key components of multiprotein complexes or principal regulators of organelle structure and function can markedly exacerbate the adverse effects of oxidative stress. For instance, oxidative damage to complex I of the electron-transport chain, which is commonly observed in PD, results in enhanced ROS generation and subsequent oxidative damage within mitochondria. This organelle damage combined with the production of nitric oxide and peroxynitrite can further result in aberrant fission of mitochondria, ultimately triggering Bax-dependent cytochrome *c* release and apoptosis (12). Another example is the *S*-nitrosylation of the E3 ubiquitin ligase, parkin, which is observed in the MPTP mouse model of PD and in patients with sporadic PD. The ensuing loss of the parkin E3 ligase activity results in accumulation of α-synuclein, which, in turn, compromises function of the ubiquitin–proteasome degradation pathway. Dysfunction of the proteasome then acts as a positive-feedback mechanism to exacerbate further the accumulation and aggregation of α-synuclein (6). In a similar manner, the autophagy–lysosomal degradation pathway is also susceptible to oxidative damage. In AD, the toxic Aβ peptide may cause direct intralysosomal oxidative stress and subsequent lysosomal membrane permeabilization, causing the release of proteolytic cathepsins into the cytoplasm (13). In addition, the loss of lysosomal integrity will compromise the degradation of proteins through the autophagy pathway and may ultimately exacerbate the accumulation of toxic protein aggregates. Collectively, these findings indicate that the inhibition of protein oxidation will likely be neuroprotective, not only by preserving the function of unique proteins, but also by

Department of Biological Sciences and Eleanor Roosevelt Institute, University of Denver, Denver, Colorado.

maintaining the functions of key degradation pathways like the ubiquitin–proteasome and lysosomal–autophagy systems. In addition, reducing protein oxidation will help maintain mitochondrial structure and function.

Novel Antioxidant Strategies for Neuroprotection

Antioxidant supplementation and mitochondrial targeting

For the past several decades, antioxidant supplementation has been intensely investigated as a possible therapeutic avenue for various neurodegenerative diseases. Antioxidants such as vitamin E, vitamin C, *N*-acetylcysteine, and glutathione (GSH) have been tested in a number of clinical trials for neuroprotection. Unfortunately, most of these trials have shown little to no benefit to patients (reviewed in 3). Recently, research has focused on novel ways to target antioxidants to particular organelles (*e.g.*, mitochondria) that are known to generate high levels of ROS. For instance, mitochondrial-targeted antioxidant peptides, vitamin E, and ubiquinone (MitoQ) are all being actively studied for their neuroprotective properties (10, 15, 19). In addition, the development of catalytic antioxidants, manipulation of endogenous antioxidant pathways, and characterization of natural-product ("nutraceutical") antioxidants are all active research areas for neuroprotection.

Catalytic antioxidants

Novel chemical compounds that mimic the catalytic activities of either superoxide dismutase or catalase are currently being evaluated in multiple *in vitro* and *in vivo* animal models of neurodegeneration (7). These compounds have a distinct advantage over most common antioxidants, in that they act catalytically to detoxify free radicals rather than acting stoichiometrically merely to scavenge ROS. Several chemical classes of catalytic antioxidants are under development, and their promising effects in animal models suggest that they may produce tangible benefits to patients in clinical trials of neurodegeneration.

Modulation of endogenous antioxidant transcriptional regulators

Endogenous antioxidant pathways are tightly regulated at the transcriptional level. One of the principal transcription factors that modulates endogenous antioxidant expression is nuclear factor E2–related factor 2 (Nrf2). Nrf2 turns on the expression of a diverse array of antioxidant, detoxification, and cytoprotective genes, including those involved in GSH synthesis (reviewed in 2 and 4). Because of this pleiotropic response downstream of Nrf2 activation, strategies to stimulate Nrf2 transcriptional activity may be beneficial in neurodegeneration. Several approaches could be used to stimulate Nrf2 activity *in vivo*, such as administration of small-molecule activators of Nrf2, overexpression of Nrf2, or disruption of the activity of Keap1, an E3 ligase and endogenous repressor of Nrf2 (2). Intriguingly, the ability of Nrf2 overexpression to provide neuroprotection may not even require Nrf2 delivery to neurons; rather, Nrf2 expression in astrocytes appears to be a viable strategy for protecting neighboring neurons (2).

Another transcriptional regulator of endogenous antioxidant defense programs is peroxisome proliferator–activated receptor-γ co-activator-1α (PGC-1α). PGC-1α is a pleiotropic transcriptional coactivator that has some minimal overlap with Nrf2 in the array of antioxidant genes that it regulates. However, PGC-1α also modulates the expression of a number of antioxidant proteins within mitochondria (*e.g.*, specific peroxiredoxin isoforms) that are apparently not regulated by Nrf2 (4). The induction of PGC-1α activity, by using strategies similar to those outlined earlier for Nrf2, may result in an antioxidant response that is complementary to that observed with Nrf2 activation (4). Thus, one can envision a novel approach of stimulating both Nrf2 and PGC-1α transcriptional pathways as a potential combination therapy for neurodegeneration.

Natural products ("nutraceuticals")

Natural products in the diet have recently gained significant attention as possible therapeutic candidates for neurodegeneration. These nutraceuticals are found in a variety of foods and spices and are chemically defined as flavonoids, polyphenols, catechins, curcuminoids, *etc.*, as well as organosulfur compounds. Some of these compounds provide neuroprotection directly *via* free radical scavenging, whereas others, such as the polyphenol resveratrol and the organosulfur compound sulforaphane, activate Nrf2-dependent transcription of endogenous antioxidant genes. An example of the latter strategy to protect dorsal root ganglion neurons from hyperglycemia-induced oxidative stress is reported by Vincent *et al*. (17) in the current issue. In addition, some natural polyphenols, such as the green tea catechin, epigallocatechin 3-gallate (EGCG), show an innate capacity to accumulate in mitochondria and protect neurons from mitochondrial oxidative stress, which is a prominent cause of neurodegeneration (14). Finally, some natural polyphenols like curcumin have been chemically modified to produce derivatives that display enhanced antioxidant, antiinflammatory, and neuroprotective properties (11). These observations demonstrate that nutraceuticals provide an abundant source of potential therapeutic agents that could be used to mitigate oxidative stress and inflammation in neurodegenerative diseases.

Future Directions

Over the past several decades, substantial evidence has accumulated showing a causative role for oxidative stress in neurodegeneration. This pathologic process occurs primarily as a consequence of aging, but additional genetic and environmental factors clearly contribute to the aberrant generation of ROS and RNS. Although past trials with traditional antioxidants have produced relatively little benefit in patients, novel approaches to enhance the therapeutic potential of antioxidants are actively being investigated. Among these approaches is the modification of natural antioxidants either selectively to target them to mitochondria or to enhance their free radical–scavenging and antiinflammatory activities. In addition, the discovery and development of catalytic antioxidants with increased potency and penetration into the CNS are ongoing. Finally, a strategy that may hold significant promise for neurodegeneration is the manipulation of transcriptional regulators of the endogenous antioxidant response, such as Nrf2 and PGC-1α. In addition to the further discovery and development of novel antioxidant

strategies like those mentioned, future research also is necessary to identify key molecular targets and pathways that are substrates for oxidative stress and that must be preserved to alter the course of neurodegenerative disease. The identification of these oxidative stress–sensitive molecules may provide new opportunities for specifically targeting critical proteins or pathways to protect them selectively from oxidative damage. Future clinical trials using novel antioxidant approaches for neurodegeneration are on the horizon, and the neuroscience community and patients with these debilitating diseases are anxiously hoping for positive outcomes.

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Address reprint requests to:
Daniel A. Linseman, Ph.D.
University of Denver
Department of Biological Sciences and Eleanor Roosevelt Institute
Seeley G. Mudd Science Bldg., Rm. 130
2101 E. Wesley Ave.
Denver, CO 80208

E-mail: daniel.linseman@du.edu

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