Therapeutic Potential of the Activators of the Nuclear Factor Erythroid 2-Related Factor 2–Antioxidant Response Element Pathway in Brain Disorders

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Oxidative stress is recognized as an important mediator of brain disorders. Nevertheless, there are few antioxidants approved for brain diseases. There are two types of mechanisms as antioxidant systems in vivo, antioxidants and antioxidant enzymes. Antioxidants are consumed by the reaction with reactive oxygen species. Thus, it is important to maintain high concentrations at the requisite site. On the other hand, antioxidant capacity is maintained for around a half-day to one day once antioxidant enzymes are induced. Therefore, low molecular-weight compounds that could induce antioxidant enzymes are considered to be suitable for the treatment and prevention of brain diseases. The nuclear factor erythroid 2-related factor 2 (Nrf2)–antioxidant response element (ARE) pathway is known as a system for inducing these antioxidant enzymes. Here, the potential for low molecular-weight compounds capable of activating the Nrf2–ARE pathway to become therapeutic agents for brain diseases is discussed.

Key words oxidative stress; brain disorder; neurodegenerative disease

1. INTRODUCTION

The problem of higher brain dysfunction due to neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease (PD), and cerebral ischemia is characterized by neuronal death in specific areas of the brain through processes such as apoptosis and necrosis. After the morbidity of these diseases, it is very difficult to cure completely. Thus, it is necessary to manage them from the viewpoint of preventive medicine in order to overcome brain diseases. Since the symptoms gradually progress over a long period of several years or more, it is important to delay or prevent the onset of these diseases not only by medication but also by the auxiliary use of food with neuroprotective action.

Oxidative stress is recognized as an important mediator of brain disorders. It is known that reactive oxygen species (ROS) contribute to other known mechanisms of brain damage such as mitochondrial dysfunction and inflammation. The development of antioxidant therapy has long been required due to the role of oxidative stress in brain diseases. Historically, the development of natural and synthetic antioxidants as candidates for treatment has been difficult despite the existence of promising preclinical and epidemiological data. Currently, some drugs with antioxidant activity are in clinical and preclinical development for neurological diseases e.g., edaravone, idebenone, and dimethyl fumarate.

There are two types of antioxidant mechanisms in vivo (Fig. 1), antioxidants and antioxidant enzymes. Since many antioxidant substances are low molecular-weight compounds and these are consumed to remove ROS, it is necessary to maintain a high concentration in the affected site. On the other hand, antioxidant capacity is maintained after the expression of antioxidant enzymes. The nuclear factor erythroid 2-related factor 2 (Nrf2)–antioxidant response element (ARE) pathway is known as the in vivo system for inducing these antioxidant enzymes. Therefore, this paper discusses the possibility of treating brain diseases by activating the Nrf2–ARE pathway.

2. NRF2–ARE PATHWAY

The Nrf2–ARE pathway is a major determinant of phase II gene induction, including many antioxidant enzymes. Under physiological conditions, Nrf2 is inactivated by binding to a skeletal actin-binding protein, Kelch-like ECH-related protein 1 (Keap1). Under conditions of oxidative stress, Nrf2 is no longer sequestered by Keap1, but is transferred to the nucleus and binds to the ARE. This induces several phase II detoxification enzymes such as heme oxygenase-1 (HO-1), γ-glutamylcysteine synthetase (γ-GCS), and nicotinamide

![Antioxidants](https://via.placeholder.com/150)

Antioxidants
From diet or synthetic Low molecular weight
Vitamin C
Vitamin E
Glutathione etc.

![Antioxidant enzymes](https://via.placeholder.com/150)

Antioxidant enzymes
Protein
SOD Catalase Hemeoxygenase etc.

Fig. 1. Two Types of Antioxidant Mechanisms in Vivo

Many antioxidants are low molecular-weight compounds. Antioxidant enzymes are proteins that have antioxidant capacity.
adenine dinucleotide phosphate (NAD(P)H):quinone oxidoreductase.

Recent research has suggested Keap1-independent Nrf2 activation models. Glycogen synthase kinase (GSK)-3β phosphorylates the Neh6 domain of Nrf2 in these models. Phosphorylated Neh6 on Nrf2 is recognized by an E3 ubiquitin ligase. Thus, GSK-3β-mediated phosphorylation of Neh6 causes ubiquitination and degradation of Nrf2 in place of Keap1. However, further investigation is required to confirm the contribution of this pathway to antioxidant enzyme expression.

A number of proteins are involved in the regulation of the Nrf2–ARE signaling pathway. Some proteins exert their functions by directly modifying the Keap1-Nrf2 complex in the cytoplasm, while other proteins function in the nucleus. An example of cytosolic modification suggests that p21cip1/WAF1 binds directly to Nrf2. Competition between p21cip1/WAF1 and Keap1 for Nrf2 binding impairs Keap1-mediated Nrf2 ubiquitination. Other proteins affect Nrf2 activity in the nucleus. For example, BACH1 has been identified as a repressor of Nrf2. Under basic conditions, BACH1 forms a heterodimer with the Maf protein, which then occupies the ARE sequence and negatively regulates several phase II genes such as HO-1. Under the conditions of oxidative stress, BACH1 is phosphorylated and exported to the cytoplasm. Thus, free Maf proteins heterodimerize with Nrf2 and induce the expression of the downstream gene. Phosphorylation is another important mechanism for regulating Nrf2-dependent gene expression. Several kinases such as protein kinase C (PKC) and phosphatidylinositol 3-kinase (PI3K) increase Nrf2–ARE transcription. Several kinases such as protein kinase C (PKC) and important mechanism for regulating Nrf2-dependent gene expression.

Studies investigating the relationship between PI3K signaling and the Nrf2 pathway showed that PI3K is located upstream of Nrf2 and its transcriptional activity in IMR-32 human neuroblastoma cells and primary cortical neuronal cultures. PKC phosphorylates Nrf2 and promotes dissociation from Keap1. Studies investigating the relationship between PI3K signaling and the Nrf2 pathway showed that PI3K is located upstream of Nrf2 and its transcriptional activity in IMR-32 human neuroblastoma cells and primary cortical neuronal cultures; although the detailed mechanism is not yet fully understood.

Collectively, the ubiquitination of Nrf2 through either the Keap1-dependent or -independent pathway is a fundamental mechanism for inhibiting Nrf2. The activation of Nrf2 is initiated by the dissociation of Nrf2 from Keap1, preventing its ubiquitination and causing its translocation to the nucleus. Nrf2 finally binds to the ARE and causes phase II gene expression through exquisitely coordinated control by multiple kinases and proteins.

3. ACTIVATION OF THE NRF2–ARE PATHWAY IN BRAIN DISORDERS

3.1. Ischemic Stroke Stroke is a major cause of brain damage, and ischemic stroke is the most common type. Multiple pathological processes are involved in the progression of stroke such as excitotoxicity, oxidative stress, inflammation, and mitochondrial dysfunction. Among them, oxidative stress is one of the main factors. Nrf2 is thought to act as a compensatory mechanism for stroke. For example, Keap1 decreases 2h after reperfusion following middle cerebral artery occlusion (MCAO). In addition, antioxidant proteins downstream of Nrf2, including γ-GCS and HO-1, increased significantly after 24–72h of MCAO. The administration of Nrf2–ARE activators, such as tert-butyl hydroquinone (tBHQ) and 1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl] imidazolide, reduced infarct size after ischemia in the rodent MCAO model. Consistent with those reports, Nrf2−/− mice reperfused after MCAO showed larger infarct size. The activation of the Nrf2 pathway is important to eliminate ROS contributing to neuroprotection against ischemic brain injury. For example, Nrf2−/− mice produce more ROS in brain injury.

3.2. Parkinson’s Disease PD is a brain disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta and there are many deposits of Lewy bodies in the brain. A study using autopsy PD brain revealed that a higher level of Nrf2 is expressed in the substantia nigra neurons. Upregulation of Nrf2 in PD is thought to be a compensatory response to enhance the antioxidant defense in response to oxidative stress.

An acute PD model induced by the administration of 1-methyl-4-phenylpyridine (MPTP) exhibited a greater loss of dopamine transporters in the striatum of Nrf2−/− mice. In addition, the oral administration of 3H-1,2-dithiole-3-thione, an Nrf2 inducer, to wild-type mice prevented MPTP toxicity. The protective role of Nrf2 in PD is further supported by the clinical use of a monoamine oxidase inhibitor, deprenyl (selegiline). Recent studies have focused on the neuroprotective action of deprenyl depending on the activation of Nrf2. Nrf2 may also be involved in the neuroprotective effect of DJ-1/PARK7, which reduces the ubiquitination of Nrf2 by interfering with Keap1. In addition, tBHQ can no longer induce nuclear translocation of Nrf2 in DJ-1 knockout mice.

Therefore, Nrf2 dysfunction may be involved in the pathogenesis of early-onset familial PD associated with a DJ-1 mutation. It is conceivable that several mechanisms are involved in the useful effects of Nrf2 activation on these PD model animals. The first mechanism is toxin detoxification. It was reported that Nrf2−/− mice showed more severe neuronal loss than HO-1−/− mice in response to MPTP. This is believed to be because Nrf2 upregulates HO-1 expression as well as other phase II enzymes that may detoxify MPTP or 1-methyl-4-phenylpyridinium. The second mechanism is the antiinflammatory effect of Nrf2. The microglia have at least two different phenotypes of M1 and M2. After exposure to MPTP, both wild-type and Nrf2−/− mice showed elevated levels of the two markers of M1 microglia, cyclooxygenase-2 and inducible nitric oxide synthase. However, only Nrf2−/− mice exhibited reduced levels of FIZZ-1 (found in inflammatory zone), arginase-1, and interleukin-4, which are markers of M2 microglia.

Those results suggested that Nrf2 contributes to the reduction of inflammation and wound healing and that
Nrf2 plays an important role in the regulation of microglial dynamics.

3.3. Multiple Sclerosis Multiple sclerosis (MS) is an inflammatory autoimmune disease and typically causes lesions in the white matter of the brain and spinal cord. The exact etiology of MS has not been accepted. However, it is generally accepted that the proliferation of immune cells such as T cells infiltrating into the central nervous system damages oligodendrocytes and axons via neuroinflammation and oxidative stress.26,27) The infiltration of CD4+ T cells results in excessive activation of macrophages, microglia, and astrocytes, producing ROS and directly injuring normal tissues. Interestingly, Nrf2 can regulate the autoimmune neuroinflammatory response in an MS model. Several problems have been pointed out, but experimental autoimmune encephalomyelitis (EAE) is a widely accepted MS animal model. Both enhancement of immune cell infiltration including CD4+ T cells and CD19+ B cells and glial cell activation such as astrocytes and microglia have been suggested in Nrf2 knockout mice with EAE.28) In addition, Nrf2−/− mice are highly susceptible to lipopolysaccharide-induced neuroinflammation and show increased microglial infiltration and inflammatory mediator expression. These features were reported to be inhibited by sulforaphane.29) Research using autopsies of MS brains demonstrated that Nrf2-dependent gene expression rarely occurs in MHC class II-positive invasive macrophages and reactive astrocytes. In chronically progressive MS patients, alpha-motor neurons express higher Nrf2 levels compared with controls.27) Nrf2 was not detected in oligodendrocytes of either control white matter or MS brain tissue.30) Consistent with these findings, Johnson and colleagues reported that Nrf2 knockout mice markedly demonstrate demyelination and axonal loss in the brain.28)

Efforts have been made to treat MS by activating the Nrf2 pathway. Dimethyl fumarate (DMF) was approved in Europe and the U.S.A. as a treatment for MS based on the finding that DMF promotes Nrf2 activation via direct modification of Keap1 at cysteine residue 151.27,30) However, it was reported that DMF shows a therapeutic effect even in Nrf2−/− mice, and further research is needed on the site of action of DMF.

4. CONCLUSION

Oxidative stress is considered as a therapeutic target for neurological diseases because cells in the central nervous system are vulnerable to oxidative damage and high levels of ROS cause brain damage. The development of antioxidants is required to consider both the beneficial and harmful effects of ROS by monitoring side effects in healthy and diseased populations. In particular, it is imperative to remove only excess ROS rather than removing all, because low levels of ROS play a physiological role in cell signaling. It is important to develop reliable biomarkers such as more quantitatively measuring the oxidative stress level of each organ including the brain (Fig. 2). Using such biomarkers, it would be possible to maintain the improvement of the oxidative stress level in the brain and suppress the onset of brain diseases through preventive measures in an appropriate manner at an appropriate time. In addition to the intake of antioxidants with low molecular weight, it is also important to maintain antioxidant enzyme levels, such as by activating the Nrf2–ARE pathway.

Conflict of Interest The author declares no conflict of interest.

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