Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

Keap1-Nrf2 PPI inhibitors

Evidence Summary

Potentially safer alternative to currently available Nrf2 activators for preventing cell damage during aging. Requires further development and testing.

Neuroprotective Benefit: May help protect against oxidative stress-induced neuronal loss. Likely to be more useful as preventative than as a treatment.

Aging and related health concerns: May help protect against inflammation and oxidative stress-mediated cell damage.

Safety: Expected to have less off-target effects than electrophilic Nrf2 activators, but safety profile has not been established.

What is it? Keap1-Nrf2 protein-protein interaction (PPI) inhibitors are reversible activators of the Nrf2 antioxidant pathway. The interaction of Nrf2 with Keap1 prevents Nrf2 from activating its target antioxidant genes. All previously identified natural and synthetic activators of the Nrf2 pathway are electrophilic compounds that activate Nrf2 through irreversible covalent modification of cysteine residues on Keap1. These activators have shown beneficial effects in animals and/or humans in protection from oxidative stress damage, enhancement of mitochondrial function, and preventing/alleviating inflammation [1]. However, these compounds are not specific to Keap1-Nrf2, as they are also capable of modifying cysteine residues on other proteins, leading to unwanted side effects that mitigate their chronic use to prevent the accumulation of oxidative stress damage in the body throughout the aging process.

Keap1-Nrf2 PPI inhibitors offer a potentially safer alternative, since they are designed to be highly specific and reversible. The crystal structure of the Keap1-Nrf2 interaction interface was recently solved, and several of these PPI inhibitors have been discovered and have begun to be tested in cell and animal models [1]. Like the other known (electrophilic) Nrf2 activators, these compounds suffer from poor bioavailability, and there are extensive medicinal chemistry efforts underway to improve bioavailability. It appears likely that an acceptable candidate will be available for preclinical testing within the next couple years.

Neuroprotective Benefit: May help protect against oxidative stress-induced neuronal loss. Likely to be more useful as preventative than as a treatment.

If Keap1-Nrf2 PPI inhibitors can be developed that can potently activate Nrf2 *in vivo*, have good bioavailability, and are blood brain barrier (BBB) penetrant, they are expected to be neuroprotective in preventing future damage, but not in healing prior damage.

In vitro studies have demonstrated that Keap1-Nrf2 PPI inhibitors, MIND4 and MIND4-17, can activate Nrf2 target genes in neurons and astroglia, and reduce reactive oxygen species (ROS) in microglia [2]. MIND4 is both a SIRT2 inhibitor and Nrf2 activator. MIND4-17 is a more specific Nrf2 activator but has very poor bioavailability and is unable to penetrate the BBB. Pretreatment with MIND4-17 was able to protect human primary retinal ganglion cells from ultraviolet radiation (UVR)-induced oxidative stress damage [3], while MIND4 was protective against neurodegeneration in a mouse brain slice model of Huntington's disease [2]. Additionally, intracerebroventricular injection of the DEETGE-CAL-Tat peptide was able to protect against neuronal death and preserve cognitive function in rats following a cerebral ischemic injury [4]. These studies provide proof-of-principle that Keap1-Nrf2 PPI inhibitors have neuroprotective properties.

Aging and related health concerns: May help protect against inflammation and oxidative stress-mediated cell damage.

The limited studies conducted thus far provide proof-of-principle for the ability of Keap1-Nrf2 PPI inhibitors to protect against oxidative stress damage, impact mitochondrial function, and prevent inflammation. If compounds with good bioavailability can be developed, they may be useful in preventing the increases in vulnerability to cell stress associated with aging.

Oxidative stress protection:

Compound 7 was identified as a Nrf2 activator in a screen by GlaxoSmithKline. It was shown to protect human lung epithelial cells from oxidative stress mediated (tBHP-induced) glutathione depletion [5]. In rats it protected against glutathione depletion and monocyte infiltration in the lung following exposure to ozone. Compound 7 has poor (7%) bioavailability, but medicinal chemistry efforts are underway.

CPUY192018 promotes the induction of Nrf2 target genes in human colon cells *in vitro* [6]. In a mouse model of ulcerative colitis, pre-treatment prevented an increase in ROS levels and protected colon cells from cell death. Mice had less colonic inflammation and a lower grade of mucosal injury.

Mitochondrial function:

The Keap1-Nrf2 PPI inhibitor HB229 (PMI) was shown to induce p62 dependent mitophagy in cells *in vitro*, which was based on the ability of PMI to alter the mitochondrial redox status [7]. This effect was found to be specific for PP1 Nfr2 activators and did not occur with electrophilic Nfr2 activators (such as sulforaphane or dimethyl fumarate). PMI also increased mitochondrial respiratory capacity (OCR).

Anti-Inflammatory activity:

In mouse models of lipopolysaccharide (LPS)-induced inflammation, the compound 18e reduced circulating levels of pro-inflammatory cytokines [8], while the more potent compound, ZJ01, inhibited the expression of pro-inflammatory genes and the generation of ROS in the heart [9]. Treatment for 3 days showed no evidence of acute toxicity.

Safety: Expected to have less off-target effects than electrophilic Nrf2 activators, but safety profile has not been established.

Keap-Nrf2 PPI inhibitors are expected to have a better safety profile than electrophilic Nrf2 activators, however, they could potentially increase cancer risk. Further studies will need to be performed to establish the safety and tolerability of these compounds.

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