PERSPECTIVE

Neuroprotective effect of antioxidant compounds

Neurodegenerative diseases affect millions of individuals worldwide. It has been estimated that the number of patients affected by neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), traumatic brain injury (TBI), stroke and amyotrophic lateral sclerosis (ALS) will increase over time, due to the growing size of the elderly population (Willis, 2015). Currently AD affect 5.3 million people in the US and ~44 million people worldwide; PD affect 1.0 million people in the US and 7–10 million people worldwide; HD affect 30,000 people in the US and 100,000 people worldwide; TBI affect 1.4 million people in the US and 5.3 million people worldwide; Stroke affect 795,000 people in the US and 15.0 million people worldwide and finally ALS affect 12,000–30,000 people in the US and 450,000 people worldwide. Although a number of FDA approved drugs for these diseases have been used, they have been shown to produce diverse side effects and yield relatively modest benefits. Therefore, to surpass these limitations of current therapeutics, extensive research and development are underway to find drugs that are effective with less or no undesirable side effects.

Oxidative stress: Free radicals, including reactive oxygen species (ROS) and nitrogen species (RNS), are highly reactive molecules generated predominantly during cellular respiration and normal metabolism imbalance between cellular production of free radicals and ability of cells to defend against them. This phenomenon is referred to as oxidative stress (OS) which triggers ROS and RNS accumulations. ROS/RNS accumulations are implicated in a wide array of human diseases, particularly in neurodegenerative diseases (*e.g.*, AD, PD, HD, TBI, ALS, stroke and TBI) which leads to cerebral palsy. ROS/RNS have been linked to aging (Harman et al., 1956) tissue degeneration and cell death (Dixon et al., 2012; Skouta et al., 2014).

While ROS are derived from the reduction of molecular oxygen, and include superoxide anion radical (O^{2-}•), singlet oxygen (1O2), hydrogen peroxide (H_2O_2), lipid peroxides (R-O-OH) and the highly reactive hydroxyl radical (•OH), RNS are mainly derived from the reduction of nitrate (NO^{3-}) and nitrite (NO^{2-}) to nitric oxide (NOx). Free radicals (ROS/RNS) normally exist in all aerobic cells in balance with antioxidants. When this critical balance is disrupted because of free radicals, antioxidants depletion, or both, oxidative stress occurs.

Neuroprotection of oxidative stress using small molecules: While the mechanisms of these diseases are still not well understood, there is evidence for a possible therapeutic role for drugs with natural and non-natural anti-oxidative properties. Natural antioxidant compounds such as curcumin, resveratrol and epigallocatechin-3-gallate are class of compounds abundant in plants (Kim et al., 2010). Non-natural antioxidant compounds are class of compounds such as butylated hydroxyanisole, tert-butylhydroquinone and ferrostatin-1 (Skouta et al., 2014) created from a well-defined synthetic route.

Small molecules, bearing antioxidant properties, originated from plant extracts with neuroprotective effect were reviewed in the literature and will not be covered in the current manuscript. Instead and as case-study of this perspective, we will focus only on the synthesized small molecule named ferrostatin-1 (Fer-1). Fer-1 was recently identified as a potent antioxidant small molecule that was able to inhibit a non-apoptotic cell death named ferroptosis. Ferroptosis cell death involved the generation of oxidative stress particularly lipid peroxide in human fibrosarcoma HT1080 cancer cells.

It was reported that Fer-1 prevents glutamate-induced neurotoxicity in a model of organotypic hippocampal slice culture (OHSC) (Dixon et al., 2012). In this assay, it was hypothesized that Fer-1 compound is capable to act as a neuroprotective in a model of neurodegeneration such as stroke. This hypothesis was tested by using a rat OHSC model that closely resembles the hippocampus *in vivo* by preserving the integrity of neuronal connections, both inhibitory and excitatory. OHSCs were treated with a lethal excitotoxic stimulus (5 mM L-glutamate, 3 hours) that mimics the consequences of stroke. These slices were co-incubated with glutamate and vehicle alone or with glutamate plus Fer-1 (2 μM), the iron chelator ciclopiroxolamine (CPX) at 5 μM), or as a positive control, the N-methyl-D-aspartate (NMDA) receptor antagonist dizocilpine maleate (MK-801, 10 μM). The effects of these compound treatments on propidium iodide (PI) uptake as an indicator of cell death 24 hrs after the end of glutamate treatment in the CA3 field of the hippocampus was analyzed. Focusing on the compound treatment effect, Bonferroni post tests indicated that glutamate induced significant cell death in the CA3 region of the brain and that this death was attenuated significantly and to an almost identical extent by cotreatment with Fer-1, CPX, or MK-801 (Dixon et al., 2012).

Fer-1 analog with improved stability and potency was successful at decreasing cell death in an in vivo model of renal tubule necrosis (Linkermann et al., 2014). Based on these data, It was hypothesized that Fer-1 would be effective at preventing other forms of cell death involving oxidative stress. For example, Fer-1 was protective in cellular models of HD (Skouta et al., 2014) and PD (Kabiraj et al., 2015). First, Fer-1 was tested, in rat corticostriatal brain slices of HD model, for its ability to prevent the cell death induced by the expression, *via* biolistic transfection, of a huntingtin (htt) exon 1 fragment with a pathogenic repeat (73Q) (mN90Q73), along with yellow fluorescent protein (YFP) to mark transfected neurons. Slices were treated with DMSO (vehicle control), a positive control death inhibitor combination of the adenosine A2A receptor antagonist KW-6002 (KW, 50 μM) and the JNK inhibitor SP600125 (SP, 30 μM), or Fer-1 at increasing concentrations (1 nM to 1 μM). Four days later, the number of healthy medium spiny neurons (MSNs) was quantified. A significant increase in the number of healthy MSNs was observed upon Fer-1 treatment at 10

nM, 100 nM, and 1 μM. Moreover, with 1 μM treatment of Fer-1, the number of healthy MSNs was statistically indistinguishable from both the YFP (no htt) control and the control inhibitor combination (KW + SP) (Skouta et al., 2014). Second, the neuroprotective role of Fer-1 under rotenone-induced oxidative stress in dopaminergic neuroblastoma cells (SH-SY5Y) was evaluated. Rotenone was used, in this assay, in order to mimic the oxidative stress and PD model in dopaminergic neuroblastoma cells (SH-SY5Y). Rotenone is a one of the known radical species generator that triggered the production of NOx in mitochondria *via* apoptosis (Tan et al., 1998). Under rotenone treatment an increase of reactive species occurs, which causes mitochondria dysfunction and triggered an imbalance of free radicals and antioxidant defenses, protein synthesis, folding, modification, trafficking and degradation that affect the endoplasmic reticulum (ER) proteins including PDI (Grek et al., 2014). PDI proteins are playing crucial roles in maintaining appropriate protein folding (Townsend et al., 2009). Under nitrosative stress, an excess of nitric oxide (NO) radical species, induced a chemical reaction between the PDI and nitric oxide. This chemical reaction generated a covalently bound formation of the S-nitrosyl of PDI cysteines which induced aggregation of Parkinsonian biomarkers (Uehara et al., 2006) which eliminates its ability to (i) contribute in the oxidoreductase mechanism via the thiol-disulfide exchange reactions and (ii) diminish the neuronal cell death triggered by protein misfolding. First the intrinsic antioxidant potential of Fer-1 was evaluated under cell-free conditions. Fer-1 scavenged the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) at 82%. Second, Fer-1 was not toxic toward the SH-SY5Y cells at concentrations up to 12.5 μM. Third, Fer-1 compound showed the ability to reduce both RNS and ROS intracellular levels provoked by rotenone insult in SH-SY5Y cells. Fourth, the cleavage of Poly (ADP-ribose) polymerase-1 (PARP-1), a marker protein for apoptotic activation, was assessed by immuno-blotting technique. Fer-1 showed protective properties of PARP-1 cleavage under rotenone treatment. This data highly suggest that Fer-1 is able of protecting the cells from controlled death by reducing the ER stress level. Fifth, Fer-1 compound suppressed rotenone induced activation of apoptotic pathway by regulating the expression of inducible nitric oxide synthase (i-NOS). This study suggest that rotenone exposure induce excessive RNS production by over expressing i-NOS which in turn activates the catalytic carboxy-terminal domain (89 kDa) by cleaving off amino-terminal DNA binding domain (24 kDa) of PARP-1 leading towards apoptosis. In PD histopathology α-syn is the major constituent of Lewy body (Su et al., 2009). We finally showed that Fer-1 mitigated rotenone-induced α-syn aggregation in our created stable α-syn-expressing neuronal SHSY-5Y cell line. This result clearly indicates the efficacy of Fer-1 compound in reducing rotenone induced α-syn aggregation in SHSY-5Y cell. The cause and effect relationship between reactive species and α-syn aggregate formation is still unknown.

Summary: Identifying new therapeutic strategies capable of modifying the course of neurodegenerative diseases are currently one of the major goals for the researchers of this field. Developing novel pharmaceutical compounds bearing antioxidant properties will significantly enhance our understanding of their roles against radical species in biological systems. It will help us discover powerful compounds that eventually help patients suffering from diseases that involve radical species production. Eventually, these may lead to the design of novel therapeutic strategies, offer insight into neurodegenerative diseases, and lead to more effective treatments that will positively impact clinic outcomes.

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