Neuroscience 332 (2016) 191–202

A COMBINATION OF AN IRON CHELATOR WITH AN ANTIOXIDANT EFFECTIVELY DIMINISHES THE DENDRITIC LOSS, TAU-HYPERPHOSPHORYLATION, AMYLOIDS-β ACCUMULATION AND BRAIN MITOCHONDRIAL DYNAMIC DISRUPTION IN RATS WITH CHRONIC IRON-OVERLOAD

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Abstract—Iron-overload can cause cognitive impairment due to blood–brain barrier (BBB) breakdown and brain mitochondrial dysfunction. Although deferiprone (DFP) has been shown to exert neuroprotection, the head-to-head comparison among iron chelators used clinically on brain iron-overload has not been investigated. Moreover, since antioxidant has been shown to be beneficial in iron-overload condition, its combined effect with iron chelator has not been tested. Therefore, the hypothesis is that all chelators provide neuroprotection under iron-overload condition, and that a combination of an iron chelator with an antioxidant has greater efficacy than monotherapy. Male Wistar rats (n = 42) were assigned to receive a normal diet (ND) or a high-iron diet (HFe) for 4 months. At the 2nd month, HFe-fed rats were treated with a vehicle, deferoxamine (DFO), DFP, deferasirox (DFX), n-acetyl cysteine (NAC) or a combination of DFP with NAC, while ND-fed rats received vehicle. At the end of the experiment, rats were decapitated and brains were removed to determine brain iron level and deposition, brain mitochondrial function, BBB protein expression, brain mitochondrial dynamic, brain apoptosis, tau-hyperphosphorylation, amyloid-β (Aβ) accumulation and dendritic spine density. The results showed that iron-overload induced BBB breakdown, brain iron accumulation, brain mitochondrial dysfunction, impaired brain mitochondrial dynamics, tau-hyperphosphorylation, Aβ accumulation and dendritic spine reduction. All treatments, except DFX, attenuated these impairments. Moreover, combined therapy provided a greater efficacy than monotherapy. These findings suggested that iron-overload induced brain iron toxicity and a combination of an iron chelator with an antioxidant provided a greatest efficacy for neuroprotection than monotherapy.

Key words: iron-overload, iron chelator, combined therapy, dendritic spine, mitochondrial dynamic.

INTRODUCTION

Iron-overload condition has commonly known to induce cellular toxicity by damaging the cellular compartments, such as DNA and mitochondria, as well as cause the membrane lipid peroxidation (Eaton and Qian, 2002). Moreover, the excessive amounts of iron in the brain have been shown to be associated with neurodegeneration (McNeill and Chinnery, 2011). A previous study demonstrated that the cognitive impairment following chronic iron-overload condition occurred by causing the breakdown of blood–brain barrier (BBB), impairing brain mitochondrial function leading to brain apoptosis (Sripetchwandee et al., 2014a). However, other possible underlying mechanisms regarding the cognitive decline under this condition including loss of dendritic spine density and impaired mitochondrial dynamics have not been investigated.

Dendritic spine density plays a fundamental role in the communication between neurons via synaptic transmissions of both electrical and chemical synapses and is responsible for cognitive function (Smrt and Zhao, 2010). A reduction in dendritic spine density has been shown to be associated with cognitive decline (Sripetchwandee et al., 2014b). Although it has been proposed that dendritic spines play a role in cognitive function, the effect of the iron-overload condition on dendritic spine density has not been investigated.

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Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; Aβ, amyloid-β; BBB, blood–brain barrier; DFO, deferoxamine; DFP, deferiprone; DFX, deferasirox; Drp1, dynamin-related protein 1; HFe, high-iron diet; Mfn2, mitofusin2; NAC, n-acetyl cysteine; ND, normal diet; NTBI, non-transferrin bound iron; ROS, reactive oxygen species.

http://dx.doi.org/10.1016/j.neuroscience.2016.07.003

The development of Alzheimer's disease (AD)-like pathological changes including the amyloid-β (Aβ) accumulation and tau-hyperphosphorylation are positively associated with cognitive impairment (Colijn and Grossberg, 2015). An Aβ aggregation has been related with brain oxidative stress (Misonou et al., 2000; Butterfield and Boyd-Kimball, 2004; Bartley et al., 2012) and its accumulation of Aβ impaired the mitochondrial electron transport chain via the induction of free radical generation, leading to the loss of ATP production, increased oxidative stress, promoted neurofibrillary tangles (NFTs) formation, and further caused the neuronal death (Yao et al., 2005; Dumont et al., 2011). Excessive amounts of iron caused generation of reactive oxygen species (ROS) and subsequently massively increased the level of oxidative stress (Koppenol, 2001). Although the involvement of iron accumulation in AD models has been observed (Leskovjan et al., 2011), alterations of AD pathology under conditions of iron-overload have not been studied.

An abnormality in mitochondria, including increased mitochondrial fragmentation, is an important factor that related with the dysfunction of mitochondria as well as cell death in aging and neurodegenerative diseases (Navarro and Boveris, 2010). It has been shown that a balance of mitochondrial dynamics, including mitochondrial fusion and fission, reflected in both of mitochondrial morphology and number (Westermann, 2010), and that this process plays an important role in cell life and death (Perfettini et al., 2005). Normally, the balance of mitochondrial fusion and fission is needed to maintain the mitochondrial shape, distribution and connectivity as well as their functions. Mitochondrial fusion is required for the expansion of the mitochondrial networks in cells which are an important factor in several processes such as a calcium signaling in cells and in certain developmental processes (Chen et al., 2003; Szabadkai et al., 2006). In contrast, mitochondrial fission is important to produce various in both morphological and functional of mitochondria (Ishihara et al., 2009). Although a recent study has shown that the exposure of iron treatment in rats during neonatal period resulted in changes in the expression of both protein and mRNA which are involved with mitochondrial fusion and fission mechanisms in the brain (da Silva et al., 2014), the effects of chronic iron-overload condition induced by long-term high-iron diet (HFe) consumption on brain mitochondrial dynamics in adult rats have not yet been investigated.

Iron chelation therapy has been used in practice to prevent tissue iron toxicity (Poggiali et al., 2012). The currently available iron chelators are deferoxamine (DFO), deferiprone (DFP) and deferasirox (DFX). These iron chelators provide therapeutic protective effects against cellular iron damage by reducing plasma non-transferrin bound iron (NTBI), cellular iron accumulation, and cell death in association with the improvement of cell survival and the maintenance of iron stores (Borgna-Pignatti and Marsella, 2015). However, the comparative efficacy of these iron chelators in the brain under iron-overload conditions has not yet been investigated. Our recent study also indicated that either DFP or an antioxidant, n-acetyl cysteine (NAC) attenuated brain iron toxicity under conditions of iron-overload. In addition, combined DFP + NAC therapy has been shown to restore brain mitochondrial function and cognitive function after impairment caused by iron-overload conditions (Sripetchwandee et al., 2014a). However, the effects of these iron chelators, NAC and a combination of an iron chelator with NAC on dendritic spine density, AD pathology and brain mitochondrial dynamics under conditions of iron-overload have not been demonstrated.

In this study the following hypotheses were tested: (1) An iron-overloaded condition decreases the dendritic spine density, increases AD pathology and induces an imbalance of brain mitochondrial dynamics; (2) pharmacological intervention with iron chelators: DFO, DFP, DFX as well as an antioxidant: NAC exerts equally protective effects on the brain under conditions of iron-overload and (3) combined therapy with an iron chelator and NAC in an iron-overloaded condition provides greater efficacy than monotherapy in preservation of dendritic spine density, prevention of the occurrence of AD pathology and restoration of the brain mitochondrial dynamic.

EXPERIMENTAL PROCEDURES

Experimental protocols

All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) at the Faculty of Medicine, Chiang Mai University. Young adult male Wistar rats weighing 180–200 g (n = 42) were obtained from the National Laboratory Animal Center, Salaya Campus, Mahidol University, Thailand. All rats were housed in an animal holding room under controlled conditions (20–22 °C, 50 ± 10% humidity) and lighting (12-hour day/night cycle) with free access to drinking water. After acclimatization for 1 week, then young adult rats were divided to receive either a normal diet (ND) or a HFe to induce an iron-overload condition (0.2% ferrocene/kg of diet) for 4 months with *ad libitum*. Moreover, body weight and food intake from all rats were record weekly. At 2 months into the experiment, ND-treated rats were given with a 0.9% of normal saline solution (NSS) 2 ml/kg/day as a vehicle via intragastric feeding (NDV, n = 6) while HFe-treated rats were randomly subdivided into six groups (n = 6/subgroup) to receive the assigned treatments including: 0.9% NSS 2 ml/kg/day (HFeV) and the pharmacological intervention of intraperitoneal injections of 25-mg/kg/day DFO (HFeDFO, IP), intragastric feeding of 75-mg/kg/day DFP (HFeDFP), 20-mg/kg/day DFX (HFeDFX), 100-mg/kg/day N-acetyl cysteine (HFeNAC) and a combination of 75-mg/kg/day DFP and 100-mg/kg/d NAC (HFeDFP + NAC). HFeDFO rats were injected intraperitoneally with DFO whereas the other HFe-treated rats were given their assigned treatment orally. All rats were given the assigned treatments continuously receiving their assigned diets for a further 2 months.

At the end of the experiment, rats were deeply anesthetized using 3% isoflurane and an intraperitoneal injection of 80 mg/kg thiopental and then decapitated.