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Iron accumulation in Parkinson's disease

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Abstract Although the exact cause of Parkinson's disease (PD) is still unknown, recent interest has been focused on the role of iron in the nigral cell death in PD. Several studies have shown that a selective and significant elevation in iron occurs in the substantia nigra of patients with PD. However, the mechanisms involved in iron accumulation also remain unclear. In this article, we describe recent findings regarding the mechanisms and potential toxic effects of iron accumulation in hereditary and sporadic PD and animal models of PD, including our genetic mouse model of PD. The review provides an opportunity to revisit the possible roles of iron accumulation in the pathogenic cascade(s) of PD.

Keywords Iron · Parkinson's disease · Substantia nigra · Dopaminergic neurons · 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine

Introduction

Several previous studies have reported the relationship between iron accumulation and dopaminergic cell death in the substantia nigra (SN) of the postmortem brains of patients with Parkinson's disease (PD; Earle 1968; Sofic et al. 1988; Dexter et al. 1987, 1989). However, whether the high iron content represents the initiation process or merely the result of nigral degeneration remains to be elucidated. Here, we review recent status regarding the mechanisms and potential toxic effects of iron accumulation in hereditary and sporadic PD and animal models of PD, including our data.

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Iron toxicity

Since iron is a redox-active metal and can facilitate the formation of cytotoxic hydroxyl radicals, superoxide anions, and hydrogen peroxide, accumulation of iron in the SN of patients with PD could be involved in neuronal degeneration. However, in vitro studies demonstrated that the toxic effects of iron are not limited to dopaminergic neurons; for example, treatment with large amount (>50 μM) of iron results in the death of tyrosine hydroxylase (TH)-negative non-dopaminergic cells. Hence, high concentration of free iron seems to be injurious to all types of neurons. On the other hand, significantly moderate amount (~25 μM) of iron induces preferential damage to dopaminergic neurons through interaction with dopamine inside the cells (Mochizuki et al. 1993). Dopamine can be a major source of reactive oxygen species (ROS) within the nigral cells, since oxidation of dopamine by monoamine oxidase releases hydrogen peroxide, which could in turn produce more toxic hydroxyl radicals through Fenton's reaction mediated through the action of iron (Halliwell 1989). Neuromelanin (NM) is the major iron storage in the substantia nigra dopaminergic neurons (Zecca et al. 1996, 2001, 2004a, b). Since neuromelanin chelates large amounts of iron, it prevents the hydroxyl radical production by Fenton's reaction (Zecca et al. 2008a, b). These data demonstrated that when neuromelanin is inside neurons, it is neuroprotective. On the contrary, increased tissue iron found in the parkinsonian SN may saturate iron-chelating sites on NM, and a looser association between iron and NM may result in an increased, rather than decreased, production of free radical species. It is hypothesized that this redox-active iron could be released and involved in a Fenton-like reaction leading to an increased production of oxidative radicals (Gerlach et al. 2003). Once neuromelanin

is released from dying neurons in the extracellular environment, it is able to activate microglia, increasing neuroinflammation and leading to the neuronal death (Zecca et al. 2008a, b; Zhang et al. 2011). Furthermore, overload of iron in neuromelanin typically occurs in PD where an increase of reactive/toxic iron bound to neuromelanin has been reported (Jellinger et al. 1992; Faucheux et al. 2003).

α-Synuclein plays a central role in the pathogenic cascades in hereditary and sporadic cases of PD. Indeed, α-synuclein is the major component of Lewy bodies (Spillantini et al. 1997), the pathological hallmark of PD, and point mutations in the α-synuclein gene (PARK1). Duplication or triplication of α-synuclein locus (PARK4) is potentially pathogenetic in rare cases of familial PD (Dawson and Dawson 2003; Singleton et al. 2003). On the other hand, low concentrations of certain metals, such as iron, can directly induce α-synuclein fibril formation (Uversky et al. 2001). Ostrerova-Golts et al. (2000) also reported that iron and free radical generators, such as dopamine and hydrogen peroxide, can stimulate the production of intracellular aggregates that contain α-synuclein and ubiquitin. In addition to stimulating aggregate formation, α-synuclein also appears to be neurotoxic. Iron may act in concert with α-synuclein and dopamine to induce the formation of Lewy body pathology and cell death in PD. In this regard, α-synuclein phosphorylation, which is also caused by iron, is due to CK2 upregulation (Takahashi et al. 2007).

Several studies have demonstrated the presence of brain inflammation in PD patients, with marked proliferation of reactive microglial cells (McGeer et al. 1988). Moreover, the loss of dopaminergic neurons is also associated with high levels of cytokines, ROS and nitric oxide (NO). These findings suggest that inflammatory reaction and infection can potentially be involved in the pathogenesis of PD (Furuya et al. 2004). Such inflammation reaction can also result in increased iron contents in dopaminergic neurons of PD. In addition to these neurons, the proinflammatory cytokines expressed in PD brains can also have profound and divergent effects on iron homeostasis in astrocytes and microglia (Rathore et al. 2012). In particular, proinflammatory TNF-α caused an increase in iron uptake and retention by both astrocytes and microglia, while anti-inflammatory cytokine TGF-β1 promoted iron efflux from astrocytes but caused iron retention in microglia (Rathore et al. 2012).

Iron accumulation in PD and PD animal models

Whether iron accumulation is a primary event in PD has been controversial. Several autopsy and radiological studies have reported iron storage in the SN of PD (Dexter et al. 1994; Berg 2006; Wypijewska et al. 2010). Other groups reported an increase in iron content in the early stages of PD and incidental Lewy body disease (Becker et al. 1995; Zecca et al. 2004a, b). Furthermore, locus coeruleus, a catecholaminergic brain region which degenerates in PD, has very low iron levels compared to SN (Zecca et al. 2004a, b). On the other hand, other works have shown the lack of such changes in nigral iron in pre-symptomatic PD or incidental Lewy body disease (Uitti et al. 1989; Galazka-Friedman et al. 1996). These results suggest that iron storage is not a primary event in PD. At this stage, it is difficult to determine whether excess iron is a primary cause of PD by clinical examination of PD patients. Moreover, it is possible that increased iron levels in certain brain regions could result from the altered vascularization that is observed in patients with PD (Faucheux et al. 1999).

Experimental animal models of PD

Several studies have examined iron contents in various experimental models of PD in order to determine whether iron accumulation in the SN is an early or late event. We also used a hemi-parkinsonism model in monkeys, which was prepared by unilateral injection of l-methyl-4-phenyl-l,2,3,6-tetrahydropyridine (MPTP) into the caudate or putamen, and compared iron content in the SN and other basal ganglia by immunohistochemistry (Mochizuki et al. 1994). The results showed that injection of MPTP into the caudate or putamen resulted in marked increase in ferric iron-reaction products in the ipsilateral SN pars compacta. The results indicated that injury to the nigrostriatal system following MPTP injection can induce iron accumulation in the SN. We also confirmed the expression of ferritin in the same model by immunohistochemistry using antibody against L-ferritin (Goto et al. 1996). Interestingly, there was no significant difference in the immunostaining for ferritin in the pars compacta of the SN between the injected and non-injected sides. The normal ferritin immunostaining on the MPTP-injected side suggests that iron accumulation is not related to altered metabolism of L-ferritin in this model. Temlett et al. (1994) measured the total free iron concentration using unilaterally MPTP-treated African green monkeys, which showed obvious contralateral hemiparkinsonism. They confirmed the excess iron accumulation in damaged dopaminergic neurons in MPTP-treated monkeys. He et al. (2003) also investigated changes in iron content in the SN at day 1 to month 18 after MPTP injection, and the relationship between iron accumulation and dopaminergic cell death progression in monkeys with parkinsonism induced by injection of MPTP. They demonstrated the presence of apoptosis in the ipsilateral SN at 1 day after MPTP injection, and a significant decrease in the number of TH-positive cells from 1 week onward. However, iron content was significantly increased in the ipsilateral SN from 4.5 to 18 months after MPTP injection, and the iron

increase correlated significantly with the extent of dopaminergic cell death. Dopaminergic cell death induced by MPTP administration might lead to iron accumulation in the monkey SN, and increased iron might contribute to the progression of nigral degeneration.

Iron accumulation in familial Parkinson's disease

Various genetic causes of parkinsonism have been identified. Iron accumulation in SN has been reported in several postmortem studies. Our group has also demonstrated the presence of more intense iron staining in parkin-deficit PD, PARK2, than in control subjects and sporadic cases of PD, as well as the presence of differences in the pattern of distribution of iron staining between PARK2 and sporadic PD (Takanashi et al. 2001). What is the mechanism of iron accumulation in the presence of parkin deficit? The major transport protein responsible for iron uptake is divalent metal transporter 1 (DMT1). Recent studies demonstrated that the 1B species is regulated post-translationally by degradation via the proteasomal pathway. Roth et al. (2010) demonstrated that parkin is the E3 ligase responsible for ubiquitination of the 1B species of DMT1. Parkin deficit may increase iron entry into neurons through an increase of DMT1. Jimenez Del Rio et al. (2004) also confirmed that the *parkin* mutation from PARK2 increases the susceptibility to dopamine and iron-mediated apoptosis in lymphocytes, probably due to its failure to dispose unfolded proteins provoked by oxidative stress.

PLA2G6 was reported to be the causative gene of early-onset PARK14-linked dystonia-parkinsonism. PLA2G6 encodes group VIA phospholipase A2 (calcium-independent phospholipase $A_2\beta$; $iPLA_2\beta$). The affected patients had parkinsonism, mental retardation/dementia, psychosis, dystonia, and hyperreflexia. Magnetic resonance images showed iron accumulation in the SN and striatum. PLA2G6 mutations have been detected in nearly all cases of classic infantile neuroaxonal dystrophy (INAD), but in only a small group of cases of idiopathic neurodegeneration with brain iron accumulation (Morgan et al. 2006). INAD is a severe psychomotor disorder with early onset and rapid progression of hypotonia, hyperreflexia, and tetraparesis. Spheroids are found in both the central and peripheral nervous systems in INAD, and iron accumulation in the brain is found in a subset of these patients. Beck et al. (2011) already established $PLA2G6^{-/-}$ mice as a model of INAD, and reported the presence of motor disturbances in these mice. They confirmed the presence of mitochondrial damage and spheroid formation in the motor neurons of the spinal cord in the INAD model. We also find iron accumulation in the SN of the same mice (manuscript in preparation). Genetic models of PD may enhance our understanding of the relationship between iron accumulation and dopaminergic cell death.

Conclusion

Several findings have provided a potential involvement of iron accumulation in the nigral cell death in PD. However, it has been controversial whether the iron accumulation is a primary causative event or merely a secondary change related to the dopaminergic neuronal degeneration. Several animal models including familial PD may provide mechanistic aspects of iron accumulation in the SN and pave a new way for clinical interventions.

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