The Role of Iron in Prion Disease and Other Neurodegenerative Diseases

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A growing number of neurodegenerative conditions, such as sporadic Creutzfeldt-Jakob disease (sCJD), Alzheimer’s disease (AD), and Parkinson’s disease (PD), are associated with accumulation of iron in the brain. The underlying cause is complex and only partially known. A better understanding of whether iron plays a primary or secondary role in disease pathogenesis is desirable and is likely to help in the development of disease-specific therapeutic strategies.

Is Brain Iron Dyshomeostasis Primary or Secondary to Neurodegeneration?

Iron is essential for vital metabolic processes, but unshielded iron is toxic via Fenton chemistry. Iron metabolism is, therefore, tightly regulated to maintain homeostasis at the cellular, organelle, and systemic levels. Nonetheless, iron accumulates in several brain disorders. The mechanism underlying iron-induced neurotoxicity is evident in disorders where the function of the pathogenic protein in cellular iron metabolism is known, and consequences of dysfunction or absence are predictable, as in hereditary ferritopatgy and aceruloplasminemia. Ambiguity arises for neurodegenerative conditions, such as sCJD, AD, and PD, where brain iron dyshomeostasis is associated with aggregation of proteins with poorly defined function [1]. The diverse etiology and pathophysiology of these disorders suggests that iron accumulation follows neuronal death and is therefore of little therapeutic significance. Emerging data, however, requires a reconsideration of this concept. First, principal proteins implicated in the pathogenesis of sCJD, AD, and PD participate in cellular iron metabolism, and their aggregation induces specific changes in the expression of iron modulating proteins in the brain and cerebrospinal fluid (CSF), some reaching diagnostic significance [2,3]. Second, sequestration of iron in aggregated protein complexes induces functional iron deficiency despite increased redox-active iron, escalating the neurotoxicity [4,5]. Lastly, a coping response from surviving neurons worsens the iron dyshomeostasis and associated neurotoxicity [4].

An additional layer of complexity is introduced by the secondary effects of inflammation, microgliosis, and astrocytosis that follow neuronal insult, each causing distinct changes in brain iron metabolism through specific pathways. The distinction between primary and secondary events blurs with disease progression, fueling the controversy on whether brain iron dyshomeostasis is primary or secondary to disease pathogenesis [6].

How Does the Brain Maintain Iron Homeostasis?

All brain cells are bathed in CSF that provides iron and other nutrients necessary for survival. Transport of transferrin (Tf)-iron and non-Tf-bound iron (NTBI) from the systemic circulation to the CSF across the blood brain barrier (BBB) is mediated by the transferrin receptor (TfR) and metal transporters respectively. Both Tf-iron and NTBI are in the relatively nonreactive oxidized (Fe3⁺) form, requiring reduction to Fe2⁺ by ferrireductase (FR) proteins for transport across lipid bilayers. Known FR proteins include duodenal cytochrome b (dcytb), steep 2 and 3, and the prion protein (PrPc) [1,7,8]. Known metal transporters include divalent metal transporter-1 (DMT1), Zip8, and Zip14 [1]. Transported Fe2⁺ joins the labile iron pool (LIP) of endothelial cells, is oxidized and stored by cellular ferritin, or is exported to the CSF through the coupled action of ferroportin (Fpn) and ceruloplasmin that oxidizes released Fe2⁺ to Fe3⁺ that binds CSF Tf or circulates as NTBI (Figure 1) [1].

Neurons take up both Tf-iron and NTBI, astrocytes lack TfR and utilize only NTBI, and oligodendrocytes acquire iron from Tf and ferritin. Microglia recycle iron from phagocytosed cells and internalize excess NTBI for storage (Figure 1). Individual cells maintain iron homeostasis by the coordinated effort of iron regulatory proteins (IRPs) that bind to iron responsive elements (IREs) on the transcripts of iron uptake and storage proteins, modulating their stability and translation based on cellular iron needs. Iron deficiency causes up-regulation of Tf, TfR, and DMT1, and down-regulation of ferritin. The converse occurs in iron overload [1]. This mechanism ensures iron availability without any unshielded Fe3⁺ and is especially important in the brain since iron saturation of CSF Tf is 100% as opposed to ~30% for serum Tf, leaving little buffering capacity against excess iron.

Are Prion Disorders Associated with Brain Iron Dyshomeostasis?

Prion disorders result from the change in conformation of prion protein (PrPc) to a misfolded PrP-scrapie (PrPSc) form that accumulates in the brain parenchyma. Brain iron dyshomeostasis is a prominent feature of human and animal prion disorders and has been reported by several laboratories using unrelated techniques and experimental models. Biochemical analysis of brain tissue from sCJD and scrapie-infected mouse and hamster models shows increased reactivity for redox-active iron and, paradoxically, a phenotype of neuronal iron deficiency [4,9]. Pre-mortem CSF from sCJD cases reflects these changes, allowing discrimination from other dementias with a high degree of sensitivity and specificity [2]. A systems biology approach should be used in the development of disease-specific therapeutic strategies.

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approach reveals differential expression of genes involved in iron homeostasis and heme metabolism in scrapie-infected mouse brains [10], and a molecular biology approach indicates altered expression of iron regulatory and storage proteins in distinct brain regions of scrapie-infected mice [11]. Alteration of iron metabolism is also noted in the spleen of scrapie-infected mice, the main peripheral organ that replicates and accumulates peripherally introduced PrPSc [11]. Accumulation of iron in PrPSc–ferritin aggregates in a biologically unavailable form, indicating sequestration of iron in PrPSc–ferritin aggregates and spirals into a vicious cycle. Inflammatory response to neuronal death increases cytokine release that activates astrocytes and increases microglial activity and death with the release of iron dyshomeostasis.

**What Is the Cause of Brain Iron Dyshomeostasis in Prion Disorders?**

Cumulative evidence suggests that accumulation of iron in PrPSc aggregates combined with loss of function of PrPSc in iron uptake contributes to the iron dyshomeostasis in prion disease affected brains. Thus, scrapie-infected mouse and hamster brain and spinal cord tissue show a phenotype of iron deficiency that increases with disease progression and correlates with PrPSc, indicating a causal relationship [4,9]. The likely cause is sequestration of iron in PrPSc–ferritin aggregates in a biologically unavailable form in the lysosomes of scrapie-infected cells [4,14]. These aggregates are relatively stable and co-purify from infected brain homogenates despite harsh purification conditions [4,15]. Like PrPSc, ferritin from diseased brains resists protease digestion, indicating that iron denaturation by heat and detergents [9]. Chelation of iron increases the sensitivity of PrPSc to protease digestion, indicating that iron stabilizes the structure of PrPSc [14]. Notably, PrPSc and ferritin are co-transported across intestinal epithelial cells in vitro and in vivo, suggesting clinical implications of PrPSc–ferritin aggregates beyond brain iron dyshomeostasis [15].

Additionally, conversion of PrPSc to PrPSc is likely to compromise its functional activity in cellular iron uptake, worsening the iron deficiency in diseased brains. PrPSc promotes iron uptake by providing the necessary FR activity [7], and its absence in PrPSc mice reduces the iron content of major systemic organs,
hematopoietic cells, and the brain [16]. Uptake of NTBI is affected more than TF-iron as indicated by decreased iron content of liver and bone marrow macrophages of PrP<sup>C</sup>-/- mice despite experimentally-induced systemic iron overload [7]. Reabsorption of NTBI from the glomerular filtrate is also reduced in PrP<sup>C</sup>-/- mice, suggesting a prominent role of PrP<sup>C</sup> in kidney iron metabolism (unpublished observations). Introduction of PrP<sup>C</sup> in the PrP<sup>C</sup>-/- background restores iron metabolism to wild-type levels, underscoring the functional role of PrP<sup>C</sup> in iron uptake [16]. Altered levels of iron in the brains of PrP<sup>C</sup>-/-, wild type, and PrP<sup>C</sup>-overexpressing mice as a function of PrP<sup>C</sup> expression have also been reported using X-ray fluorescence imaging, supporting these observations [17].

What Is the Cause of Brain Iron Dyshomeostasis in AD and PD?

Regional accumulation of iron has been reported in AD and PD brains and results from distinct pathogenic processes [1]. In AD, iron accumulates in amyloid plaques in association with amyloid beta (Aβ), a proteolytic product of the amyloid precursor protein (APP). Aβ binds iron with high affinity, rendering it redox-active. This causes localized accumulation of iron and aggregation of additional Aβ, amplifying the process [5]. Moreover, APP mediates the export of excess neuronal iron by stabilizing Fpn and providing the necessary ferroxidase activity and is itself regulated by cellular iron through IRP sequences in its transcript [18,19]. The development of brain iron dyshomeostasis in AD is therefore complex and involves altered functional activity of APP, sequestration of iron in Aβ aggregates, and additional processes discussed in a recent review [19]. A full understanding of the underlying mechanism, however, is lacking.

PD has a multifactorial etiology and is associated with loss of dopamine (DA)-producing neurons in the substantia nigra (SN). Accumulation of iron in PD brains has been attributed to secondary causes such as mitochondrial dysfunction and release from iron-rich DA neurons. However, up-regulation of DMT1 and down-regulation of Fpn in the SN of human cases and experimental models of PD suggests that accumulation of iron in PD is an active process, not a mere outcome of disease pathogenesis, and requires further exploration [20].

In conclusion, primary triggers that induce brain iron dyshomeostasis in sCJD, AD, and PD are specific to each disorder. Secondary events that follow neuronal injury are shared, each contributing to iron dyshomeostasis through distinct pathways (Figure 2). Ideally, therapeutic strategies should inhibit or block both processes and reduce iron-induced neurotoxicity by restoring brain iron homeostasis. This is a challenging task because most neurodegenerative conditions are initiated by multiple events, including aging, and are fueled by several pathways that converge to neuronal demise. Iron dyshomeostasis is one pathway in this continuum of events that holds therapeutic promise and requires focused investigation.

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References

1. Singh N, Halder S, Tripathi AK, Horback K, Wong J, et al. (2014) Brain iron homeostasis: from molecular mechanisms to clinical significance and therapeutic opportunities. Antioxid Redox Signal 20: 1324–1363.
2. Halder S, Beveridge J, Wong J, Singh A, Galinberter D, et al. (2013) A low molecular-weight ferroxidase is increased in the CSF of sCJD cases: CSF ferroxidase and transferrin as diagnostic biomarkers for sCJD. Antioxid Redox Signal 19: 1662–1675.
3. Oliveri S, Conti A, Lunnaceone S, Cannistraci CV, Campanella A, et al. (2011) Ceruloplasmin oxidation, a feature of Parkinson’s disease CSF, inhibits ferroxidase activity and promotes cellular iron retention. J Neurosci 31: 18568–18577.
4. Singh A, Isaac AO, Luo X, Mohan ML, Cohen ML, et al. (2009) Abnormal brain iron homeostasis in human and animal prion disorders. PLoS Pathog 5: e1000336.
5. Greenough MA, Camakaris J, Bush AI (2013) Metal dysregulation and oxidative stress in Alzheimer’s disease. Neurochem Int 62: 540–555.
6. Andersen HH, Johnsen KB, Moos T (2014) Iron deposits in the chronically inflamed central nervous system and contributes to neurodegeneration. Cell Mol Life Sci 71: 1607–1622.
7. Singh A, Halder S, Horback K, Tom C, Zhou L, et al. (2013) Prion protein regulates iron transport by functioning as a ferritinedase. J Alzheimers Dis 35: 541–552.
8. Ohgami RS, Campagna DR, McDonald A, Fleming MD (2006) The Steap proteins are metallochaperones. Blood 108: 1389–1394.
9. Singh A, Qing L, Kong Q, Singh N (2012) Change in the characteristics of ferritins induces iron imbalance in prion disease affected brains. Neurobiol Dis 45: 930–938.
10. Hwang D, Lee YI, Yoo H, Gehlenborg N, Cho JH, et al. (2009) A systems approach to prion disease. Mol Syst Biol 5: 252.
11. Kim BH, Jun VC, Jin JK, Kim JL, Kim NH, et al. (2007) Alteration of iron regulatory proteins (IRP1 and IRP2) and ferritin in the brains of scrapie-infected mice. Neurosci Lett 422: 158–163.
12. Huzarzewicz RL, Medena S, Robertson C, Parchalnik D, Booth SA (2011) Transcriptional modulation in a leukocyte-depleted splenic cell population during prion disease. J Toxicol Environ Health A 74: 1504–1520.
13. Ferrario S, Halldin J, Bedesca K, Lund T (2005) Changed iron regulation in scrapie-infected neuroblastoma cells. Brain Res Mol Brain Res 133: 266–273.
14. Basu S, Mohan ML, Luo X, Kundu B, Kong Q, et al. (2007) Modulation of protease K-resistant prion protein in cells and infectious brain homogenate by redox iron: implications for prion replication and disease pathogenesis. Mol Biol Cell 18: 3302–3312.
15. Mishra RS, Basu S, Gu Y, Luo X, Zhou WQ, et al. (2004) Protease-resistant human prion protein and ferritin are co-transported across Caco-2 epithelial cell: implications for species barrier in prion uptake from the intestine. J Neurosci 24: 11280–11290.
16. Singh A, Kong Q, Luo X, Petersen RB, Meyerzon H, et al. (2009) Prion protein (PrP) knock-out mice show altered iron metabolism: a functional role for PrP in iron uptake and transport. PLoS ONE 4: e6115.
17. Pushke MJ, Pickering JJ, Martin GR, Tsutsui S, Jurik FR, et al. (2011) Prion protein expression level alters regional copper, iron and zinc content in the mouse brain. Metallomics 3: 206–214.
18. Bandypadhyay S, Cahill C, Balleridet A, Huang C, Lahiri DK, et al. (2013) Novel 5’ untranslated region directed blockers of iron-regulatory protein-1 dependent amyloid precursor protein translation: implications for Down syndrome and Alzheimer’s disease. PLoS ONE 8: e65978.
19. Wong BX, Duce JA (2014) The iron regulatory capability of the major protein participants in prevalent neurodegenerative disorders. Front Pharmacol 5: 61.
20. Salazar J, Mena N, Hunot S, Pujol A, Alvarez-Fischer D, et al. (2008) Divalent metal transporter 1 (DMT1) contributes to neurodegeneration in animal models of Parkinson’s disease. Proc Natl Acad Sci U S A 105: 18578–18583.