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Iron: A New Target for Pharmacological Intervention in Neurodegenerative Diseases

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Iron (Fe) is an essential element that is imperative for the redox-driven processes of oxygen transport, electron transport, and DNA synthesis. However, in the absence of appropriate storage or chelation, excess-free Fe readily participates in the formation of toxic-free radicals, inducing oxidative stress and apoptosis. A growing body of evidence suggests that Fe may play some role in neurodegenerative diseases such as Huntington disease, Alzheimer's disease, Parkinson's disease, and particularly Friedreich's ataxia. This review examines the role of Fe in the pathology of these conditions and the potential use of Fe chelators as therapeutic agents for the treatment of neurodegenerative disorders. Consideration is given to the features that comprise a clinically successful Fe chelator, with focus on the development of ligands such as desferrioxamine, clioquinol, pyridoxal isonicotinoyl hydrazone, and other novel aroylhydrazones.
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Iron (Fe) is arguably one of the most important transition trace metals of the body. Iron-containing proteins facilitate key reactions involved in oxygen transport, electron transfer, and DNA synthesis.[1] The unique environment of the brain harbors additional Fe requirements to necessitate the synthesis of myelin and neurotransmitters.[2] Therefore, disturbed Fe metabolism has implications on many cellular processes given the crucial catalytic and regulatory roles of Fe.

The catalytic ability of Fe stems from its unique redox properties, which, if not managed appropriately, have the potential to impart toxicity. As such, the body has developed specialized mechanisms and molecules to maintain Fe in a safe, nonactive form until it is required for metabolism. However, under conditions of excess and/or dysfunctional regulation, its strong redox activity in both its ferrous (Fe^{II}) and ferric (Fe^{III}) states lends its participation in Fenton chemistry, leading to the generation of reactive oxygen species (ROS). Free-radical–related oxidative stress causes considerable damage to DNA, lipids, and proteins, culminating in cell death.

Many studies have highlighted the pathological involvement of Fe accumulation and Fe-related oxidative stress in neurodegenerative diseases such as Huntington disease (HD), Alzheimer's disease (AD), Parkinson's disease (PD), and Friedreich's ataxia (FA).[3-9] The involvement of Fe and its relatives, copper (Cu) and zinc (Zn) in the aforementioned disorders, provides a rationale for the development of metal-binding drugs (chelators) as viable new therapeutic strategies. This review discusses the alterations of Fe metabolism in neurodegenerative disease and the therapeutic potential of a range of chelators.

Iron Metabolism

To complement the discussion of Fe, its dyshomeostasis in neurodegenerative states, and the synthesis of Fe chelators to counteract these conditions, it is necessary to first introduce the basis of cellular Fe trafficking and metabolism.

Under normal circumstances, the movement of Fe throughout the body is tightly regulated, and at any time, circulating nonheme Fe (bound to the serum Fe transport protein, transferrin) represents only a small percentage of the body's total Fe stores (approximately 0.1%).[10,11] Transferrin (Tf) is a serum glycoprotein that binds 2 atoms of Fe for donation to cells by binding to the specific transferrin recep-

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tor 1 (TfR1) on the plasma cell membrane.[10] The Tf-TfR1 complexes are internalized into the cell via receptor-mediated endocytosis.[10] After a reduction in endosomal pH, Tf-bound Fe is released and then transported across the endosomal membrane via the divalent metal ion transporter 1 (DMT1, previously known as Nramp2).[12,13] Although TfR1-mediated endocytosis remains the major pathway of cellular Fe uptake,[10] a number of cell types also possess nonreceptor mediated mechanisms of Fe uptake, such as pinocytosis.[10,14]

The highly evolved nature of the blood-brain barrier (BBB) is such that endothelial cell tight junctions preclude the transport of diferric Tf from the serum directly into the brain. To facilitate movement of Tf-bound Fe from the systemic circulation, endothelial cells comprising the BBB express the TfR1, which is able to bind diferric Tf.[6,10] At the luminal membrane of blood capillaries, Tf bound to the TfR1 is endocytosed into the endothelial cell, Fe dissociates from the Tf complex, and apoTf is recycled back to the cell surface.[15,16] Liberated Fe is released from the abluminal membrane by an unknown mechanism into the interstitial space of the brain and subsequently binds to brain Tf, for delivery to neurons and glial cells.[16] In addition to the traditional Tf-mediated Fe uptake pathway, it has been speculated that Fe may also have the capacity to be transported across the BBB by way of the Tf homolog melanotransferrin (MTf).[17,18] However, although MTf is found highly expressed on melanoma cells and other tumors, its levels in the brain, serum, and normal tissues are very low.[19] When the efficacy of MTf at donating Fe to the brain was directly compared with Tf, the role of the former protein in Fe transport across the BBB was found to be minimal.[20] Moreover, MTf knockout mice displayed no change in brain Fe nor whole-body Fe metabolism compared with wild-type littermates, indicating that this molecule plays a different role than Tf.[21,22]

After release from Tf and on entry into the cytosol, Fe becomes part of a poorly characterized compartment known as the intracellular labile Fe pool (LIP).[10] At present, little is known concerning the composition of the LIP, although it has been suggested to be composed of endogenous low–molecular-weight ligands such as citrate, adenosine triphosphate, or amino acids.[10] Alternatively, it could be that high–molecular-weight molecules bind and chaperone Fe for its incorporation into the mitochondrion (for heme and [Fe-S] cluster synthesis)[23] or ferritin (for storage).[10,24] The storage of Fe in ferritin and [Fe-S] clusters renders it unavailable for use in Fenton chemistry, providing the cell with protection against the damaging effects of "free Fe." Interestingly, it has been speculated that the brain possesses an additional Fe sink, that being the complex polymer pigment neuromelanin, which is specific to catecholaminergic neurons.[25] Neuromelanin has been reported to bind Fe at 2 sites and form stable complexes with Fe(III),[25,26] affording it neuroprotective qualities. In vitro, neuromelanin has been shown to act as a cytoprotective agent, attenuating oxidative damage, such as Fe-stimulated lipid peroxidation.[25,27] However, additional work on neuromelanin is essential to clarify its potential roles in brain Fe metabolism.

Tight regulatory pathways exist to control the uptake, storage, and mobilization of Fe. The control of LIP Fe levels are to some degree modulated by 2 messenger RNA (mRNA)-binding proteins known as the Fe-regulatory proteins 1 and 2 (IRPs).[10,28] Through association with Fe-responsive elements (IREs), IRP1 and IRP2 are able to posttranscriptionally regulate the expression of mRNAs of the *ferritin* and *TfR1* genes, which are essential for Fe homeostasis.[28] Such IREs are located in the 5′ or 3′-untranslated regions (UTRs) of mRNAs. For instance, in *ferritin* mRNA, the IRE is located in the 5′ UTR, whereas in *TfR1* mRNA, the IREs are located in the 3′ UTR.[28]

In response to cellular Fe levels, IRP-IRE binding acts to stabilize or inhibit mRNA translation to regulate protein expression and equilibrate Fe levels.[28] IRP1 regulation is achieved under the influence of an [4Fe-4S] cluster within the protein.[28] Under conditions of high-cellular Fe levels, the incorporation of an [4Fe-4S] cluster into IRP1 prevents its binding to the 3′ IRE in *TfR1* mRNA, decreasing its half-life and leading to decreased TfR1 translation and Tf-bound Fe uptake. Likewise, formation of the cluster prevents the binding of IRP1 to the 5′ IRE of *ferritin* mRNA, allowing translation of the mRNA on the ribosome and increasing ferritin protein levels for Fe storage.[28] Conversely, the absence of an [4Fe-4S] cluster in IRP1 in Fe-deplete cells favors its binding to the respective IREs of *TfR1* and *ferritin* mRNA to increase Fe acquisition and decrease Fe storage, respectively.[28] Unlike IRP1, IRP2 does not contain an [4Fe-4S] cluster. Rather, in Fe-replete cells, IRP2 is rapidly degraded by a proteasome-dependent mechanism.[10]

With knowledge of these regulatory functions, we can now better comprehend the consequences of Fe dysregulation such as that found in the neurologic conditions HD, AD, PD, and FA.

When Iron Roams Free: Consequences of Iron Dyshomeostasis

The ability of Fe to cycle between its 2 stable oxidation states facilitates mandatory metabolic functions via electron exchange. However, this quality also enables Fe to cause harm by way of oxidative stress. For this reason, the body tightly regulates Fe by the mechanisms described in the previous section.

Regular oxygen metabolism generates an assortment of byproducts. These include ROS such as superoxide ($O_2^{\bullet}$), hydroxyl ($OH^{\bullet}$), nitric oxide ($NO^{\bullet}$), peroxynitrite ($ONOO^{\bullet}$), and hydrogen peroxide (H_2O_2). ROS are highly reactive and potentially damaging to biomolecules. However, at low concentrations, they can act as second messengers, gene regulators, and/or mediators of cellular activation.[29] To control and balance the production of ROS, cells express a set of antioxidant and detoxifying enzymes such as superoxide dismutase, catalase, and glutathione that can manage excessive free radical generation. An imbalance between ROS production and antioxidant defense induces a state of "oxidative stress" in which ROS interact