HIGHLIGHTS

Handling iron in restorative neuroscience

The transition metal iron exerts essential roles in the central nervous system (CNS) for oxygen transport, myelin formation, and synthesis of neurotransmitters. Being redox active, iron switches between ferrous and ferric states. Switching between oxygen states also makes iron an important inducer of reactive oxygen species through the Fenton and Haber-Weiss reactions. Such reactive oxygen species are potentially damaging to nucleic acids, proteins, and fatty acids, which makes the handling of iron very important. Both of iron overload and iron deficiency are detrimental to cells of the nervous system. Iron overload increases the formation of oxidative species and thereby increasing risks for neuronal death, and regional iron accumulation in the brain is associated with neurodegenerative disorders like Alzheimer's disease and Parkinson's disease (Rouault, 2013; Andersen et al., 2014; Ward et al., 2014). Systemic iron overloading causes hemochromatosis with the surprising absence of brain impairment, which is attributed to down-regulation of the iron uptake and transport at the blood-brain barrier (BBB) (Andersen et al., 2014). Conversely, limited access to iron leads to systemic iron deficiency that may affect the iron levels in the CNS and peripheral nervous system, which can cause cell growth arrest or even cell death. This suggests that maternal iron deficiency seriously can affect the developing foetus, including the brain.

Iron is acquired from food as heme and non-heme iron. Divalent metal transporter 1 (DMT1) located on the apical membrane of enterocytes facilitates the entry of heme iron into the entero-cyte, after which ferrous iron converges to the labile iron pool inside the enterocyte. Iron is then either oxidized and stored in ferritin, or exported to the circulation by the basolaterally localized iron-transporter ferroportin. Participating in the ferroportin-mediated export, hephaestin, which is another protein exhibiting ferroxidase-activity and also expressed by enterocytes, oxidizes ferrous iron thereby facilitating the binding of ferric iron to circulating apo-transferrin to form holo-transferrin disease (Rouault, 2013; Andersen et al., 2014; Ward et al., 2014).

The entry of iron into the brain is highly regulated by transferrin receptors expressed by brain capillary endothelial cells that also denote the BBB. The cellular iron-exporter protein, ferro-portin, is also expressed at the BBB, and ferroportin is probably involved in further transport of iron into the brain, although the understanding of iron transport through the BBB remains highly debated with various hypotheses being issued (Winkler et al., 2014). The most accepted hypothesis suggests that iron is transported by a process involving receptor-mediated uptake of holo-transferrin followed by detachment of iron from transferrin within endocytotic vesicles. The holo-transferrin complex binds to the transferrin receptor expressed at the luminal side of the endothelial cells. The holo-transferrin/transferrin receptor complex undergoes endocytosis, after which the endosome acidifies, which causes the release of iron from transferrin. Metallo reductases, like STEAP 2 and STEAP3, then reduce ferric iron to ferrous iron, which is translocated across the endosomal membrane *via* DMT1 (Ji and Kosman, 2015). Iron is then exported to the interstitial fluid *via* ferroportin. Ferrous iron gets oxidized to ferric iron by another ferroxidase, glycophosphatidy-linositol (GPI)-anchored ceruloplasmin, expressed by astrocytes. Astrocytes thereby play an important role in iron regulation and detoxification. Another glial cell type, the myelin-forming oligodendrocyte, synthesize transferrin that could play an important role for binding the ferric iron. Iron uptake in neurons and most glial cells is mediated by the transferrin receptor. The high content of iron in neurons and oligodendrocytes probably reflects the high need for neurotransmitter and myelin synthesis, respectively.

Neuronal iron homeostasis: It is proposed that neurons take up iron through the transferrin receptor and DMT1, as described for iron uptake in enterocytes and endothelial cells (Andersen et al., 2014). Newly imported ferrous iron converges with the labile iron pool for immediate use in the cytosol, storage in ferritin, deliverance to the mitochondria, or cellular export *via* ferroportin (Rouault, 2013; Ward et al., 2014). The entry of iron into the mitochondria is still a controversial subject with several hypotheses currently being debated. One of the hypothesis states that metabolites cross the mitochondrial outer membrane through a voltage-dependent anion channel, while crossing the inner membrane *via* specialized carriers, such as glutaredoxin 3 and 4 (Wang and Pantopoulos, 2011; Levi and Rovida, 2015). The labile iron pool supplies cytosolic iron-dependent enzymes. Iron, which is not required immediately, is stored as ferric iron in ferritin. Ferritin is composed of a heavy chain (H) and a light chain (L), which function to oxidize and sequester free iron, and thereby preventing the formation of unwanted reactive oxygen species. Ferritin H exhibits ferroxidase activity and catalyzes the oxidation of ferrous iron to ferric iron, whereas ferritin L supports this ferroxidase activity by mineralizing iron (Levi and Rovida, 2015). The ferritin isoforms are assembled into clusters consisting of 24 molecules capable of storing up to 4,500 iron atoms. Iron is transported to the mitochondria *via* the solute carrier transporter, mitoferrin, and is mostly used for synthesis of heme or iron-sulfur clusters involved in oxidative phosphorylation (Rouault, 2013; Mancias et al., 2014). Ferritin also exists on a mitochondrial form particular in cells with a high metabolic rate like neurons (Levi and Rovida, 2015). The main function of mitochondrial ferritin is thought to prevent free iron from generation of reactive oxygen species. Ferroportin exports iron from neurons and functions to export iron through the cellular membrane, after which astrocytic ceruloplasmin oxidizes ferrous to ferric iron (Andersen et al., 2014; Ji and Kosman, 2015).

The iron responsive element/iron regulatory protein system (IRE/IRP) regulates the expression of ferritin, DMT1, transferrin receptor, and ferroportin at the post-transcriptional level and functions to stabilize or degrade mRNA (Rouault, 2013). In conditions with high neuronal iron concentration, iron-sulfur clusters are integrated in IRPs thereby blocking the binding of IREs to IRPs, which preserves ferritin - and ferritin mRNAs. Conversely, during neuronal iron deficiency, the expression of the transferrin receptor mRNA is stabilized (Jeong et al., 2011). Additionally, the hepatic hormone, hepcidin regulates the expression of ferroportin at a post-translational level, although it should be noted that the significance of hepcidin for the regulation for ferroportin in the brain in physiological conditions remains unexplained. Excessive hepcidin levels may occur in neuropathological conditions and lead to internalization and degradation of neuronal ferroportin (Urrutia et al., 2013).

Iron homeostasis in neurodegeneration and neuronal regeneration: The concentration of iron increases in brain with normal aging, but the concentration of iron in brain increases even further in pathological conditions (Andersen et al., 2014; Ward et al., 2014). The irons accumulating in the pathological brain could result from neuronal degeneration or migration of inflammatory cells containing high concentrations of iron from

the periphery into the brain. These inflammatory cells, monocytes and macrophages in particular, die out inside the brain after phagocytosis of dead or dying neurons and subsequently deposit iron (Xiong et al., 2012; Andersen et al., 2014). Iron deposition may be substantially widespread in the brain even in conditions with presumably regional neurodegeneration (Xiong et al., 2012). The integrity of the BBB is challenged in regions affected by neurodegeneration (Andersen et al., 2014). Leakiness of the BBB in the spinal cord associates with progression in amyotrophic lateral sclerosis. This disruption results in accumulation of different plasma proteins, *e.g.*, erythrocyte-derived haemoglobin and the iron-containing hemosiderin, which thereby increases the concentration of iron in the ventral horn of the spinal cord (Winkler et al., 2014).

Iron accumulation during development could also cause behavioural abnormalities and Parkinson's disease like neurochemical deficits in ageing (Wang et al., 2014). In rats with rotenone-induced Parkinson's disease, a decreased iron deposition was seen in substantia nigra, hippocampus and cerebellum after treatment with the chelating agents, deferoxamine and baicalin (Xiong, et al., 2012). This treatment also led to improved muscle endurance. Rats subjected to iron deficiency during neonatal life presumably have a decreased content of myelin in the CNS due to the impaired proliferation and differentiation of oligodendrocytes (Stephenson et al., 2014).

Inherited diseases caused by mutations in the gene encoding ferritin H cause neurodegeneration mainly in the basal ganglia leading to Parkinsonian symptoms with dysarthria (Keogh et al., 2012). Loss of IRPs also plays a role in neurodegeneration presumptively causing a condition with neuronal iron deficiency (Jeong et al., 2011). Mutant mice $Irp1^{+/-}$; $Irp2^{-/-}$ and $Irp1^{+/+}$; $Irp2^{-/-}$ exhibit dysregulation in iron homeostasis leading to iron deprivation and degeneration of spinal cord motor neurons. The iron starvation is caused by dysregulation of transferrin receptor and ferritin, as the genetic loss of IRPs results in upregulated ferritin and reduced expression of the transferrin receptor. The high level of ferritin may desolate iron and hinder the use of iron for normal metabolic purposes, while the loss of transferrin receptor leads to a diminished uptake of iron hence creating intracellular iron deficiency (Jeong et al., 2011). Hence, both iron accumulation and iron deficiency can cause neurodegeneration. Recent studies indicate a clearly beneficial effect of iron supplementation, when the neuronal need for iron is increased (Stephenson et al., 2014).

The issue of iron in regenerating neurons has not received much attention. The main observations were made by the Kreutzberg group (Graeber et al., 1989; Raivich et al., 1991). The internalization of iron was studied in regenerating motor neurons of the CNS showing that both iron uptake and transferrin receptor expression increase in somata of motor neurons after axotomy (Graeber et al., 1989). Notably, the higher expression of transferrin receptor in the soma of the regenerating motor neurons indicates that the uptake of iron took place within CNS. Transecting axons of the sciatic nerve lead to an increase in iron uptake and transferrin receptors in the endoneurium surrounding the regenerating peripheral axons, which indicates that the iron uptake is also increased in the distal tip of the regenerating axon (Raivich et al., 1991).

Iron is a prooxidant and participates in many vital cellular processes. Excess iron is clearly toxic and in conditions with mishandling of non-protein bound iron in the brain, iron exerts neurodegenerative capabilities. This is even exaggerated by the fact that iron-containing macrophages often infiltrate the brain and deposit iron as part of the neurodegenerative process. Therefore, chelation of iron is a relevant pharmacological consideration when iron overloading occurs. Conversely, iron seems to be of importance in conditions with need for regeneration of peripheral axons, and access to iron-supply may ensure adequate regeneration.

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