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Regulation of Brain Iron and Copper Homeostasis by Brain Barrier Systems: Implication in Neurodegenerative Diseases

Wei Zheng[*] and **Andrew D. Monnot**
School of Health Sciences, Purdue University, West Lafayette, Indiana, USA

Abstract

Iron (Fe) and copper (Cu) are essential to neuronal function; excess or deficiency of either is known to underlie the pathoetiology of several commonly known neurodegenerative disorders. This delicate balance of Fe and Cu in the central milieu is maintained by the brain barrier systems, i.e., the blood-brain barrier (BBB) between the blood and brain interstitial fluid and the blood-cerebrospinal fluid barrier (BCB) between the blood and cerebrospinal fluid (CSF). This review provides a concise description on the structural and functional characteristics of the brain barrier systems. Current understanding of Fe and Cu transport across the brain barriers is thoroughly examined, with major focuses on whether the BBB and BCB coordinate the direction of Fe and Cu fluxes between the blood and brain/CSF. In particular, the mechanism by which pertinent metal transporters in the barriers, such as the transferrin receptor (TfR), divalent metal transporter (DMT1), copper transporter (CTR1), ATP7A/B, and ferroportin (FPN), regulate metal movement across the barriers is explored. Finally, the detrimental consequences of dysfunctional metal transport by brain barriers, as a result of endogenous disorders or exogenous insults, are discussed. Understanding the regulation of Fe and Cu homeostasis in the central nervous system aids in the design of new drugs targeted on the regulatory proteins at the brain barriers for the treatment of metal's deficiency or overload-related neurological diseases.

Keywords

iron; copper; blood-brain barrier; blood-CSF barrier; choroid plexus; transferrin receptor or TfR; divalent metal transporter or DMT1; copper transporter or CTR1

1. Introduction

Both iron (Fe) and copper (Cu) are essential trace elements to human health. As transition metals, Fe and Cu exist in a variety of oxidation states such as Fe(II), Fe(III), Fe(VI), Cu(I), and Cu(II). The multiple oxidation states allow the metals to readily participate in oxidation-reduction reactions. Within cells, both Fe and Cu are capable of forming complexes through sulfur (S) and oxygen ligands. Because of their subtle reactivity in chemistry, Fe and Cu appear to be selected in molecular evolution to carry out a wide range of biological functions. A well-known function of Fe in the body is to transport oxygen in hemoglobin

[*]To whom all correspondence should be addressed: Wei Zheng, Ph.D., Professor of Health Sciences, School of Health Sciences, Purdue University, 550 Stadium Mall Drive, CIVL1169, West Lafayette, IN 47907, U.S.A., +1.765.496.6447 (voice), +1.765.496.1377 (fax), wzheng@purdue.edu.

and myoglobin. Fe is also required for the normal function of cytochromes, cytochrome oxidase, peroxidase, and catalase. Fe-S proteins in the mitochondrial respiration chain deliver the electrons generated from the tricarboxylic acid cycle (TCA cycle) to ADP molecules for energy production, thereby complying with the high demand of energy consumption of neuronal cells. In the brain, Fe is required not only for DNA synthesis and mitochondrial respiration, but also for the biosynthesis of neurotransmitters, axonal growth, and receptor-mediated postsynaptic signal transduction. Similarly, Cu is an integral component of various cuproenzymes, including cytochrome C oxidase, lysyl oxidase, superoxide dismutase (SOD), dopamine β-oxidase, tyrosinase and ceruloplasmin. Cu, as a free, unbound metal ion, participates in the metabolism of neurotransmitters and nerve myelination. Toxicologically, free Fe and Cu ions can readily interact with oxygen to initiate cascades of biochemical reactions leading to the production of free radicals and oxidative stress. It is for their chemical reactivity that both excess and deficiency in Fe or Cu status can be detrimental to the central nervous system (CNS). Thus, a stable homeostasis of Fe and Cu in the central milieu is essential for brain function.

The biological mechanisms that regulate Fe and Cu homeostasis take place in the processes of absorption, distribution, biotransformation, and excretion. In the CNS, these processes are located primarily in the brain barrier system, i.e., the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCB). A compromised brain barrier system, either structurally or functionally, can cause an imbalance in metals' needs and supplies in the CNS. Subsequently, a distorted brain homeostasis of either of the metal ions can gradually become an etiological factor in the initiation and/or progression of numerous neurodegenerative disorders. A vast amount of literature has implicated the direct or indirect involvement of Fe or Cu in the pathogenesis of Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, prion disease, occipital horn syndrome, and the genetic disorders Wilson's disease and Menkes' disease.

This review deals with the current understanding of the critical role of the brain barrier system in maintaining Fe and Cu homeostasis in the CNS and the relationship between distorted brain Fe or Cu regulation and the etiology of neurodegenerative disorders. The structural and functional characteristics of the brain barrier system are first introduced. The primary utilities of each metal in the human body are then briefly reviewed, which is followed by a discussion of the critical proteins and transporters involved in Fe and Cu movement at the brain barriers. To date, most studies on metal transport by brain barriers have been conducted separately in either BBB- or BCB-centered models; therefore the integrated contribution of the BBB and BCB in regulating metal transport is particularly emphasized. Finally, the diseases and disorders resulting from altered Fe and Cu transport by brain barriers, either due to endogenous genetic disorders or exogenous insults are discussed.

2. Brain Barrier Systems

The brain barrier systems are comprised of the BBB and BCB. The BBB separates the blood circulation from the brain interstitial fluid, and the BCB separates the blood from the cerebrospinal fluid (CSF) (Fig. 1). Since there is no structural barrier between the CSF and interstitial fluid, the materials present in either of these fluids can exchange freely.

The endothelial cells that line the cerebral capillaries and have the tight junctions between adjacent cells constitute the structural basis of the BBB. Thus, the BBB continues where the cerebral capillaries extend into the entire brain. In the human brain, the estimated total length of the brain capillaries is about 650 km with a surface area between 10–20 m^2 (Bradbury, 1979; Pardridge, 1990, 1991). These cerebral endothelial cells express unique

tight junction proteins, such as occludin, claudins and ZO1, which seal one adjacent cell with another. Surrounding the abluminal side of the brain capillary endothelial cells are three cell layers: (i) a basement membrane, (ii) pericytes that surround ~30% of the endothelial cell surface, and (iii) astrocyte foot processes that cover over 90% of the surface of endothelial cells and pericytes (Cecchelli et al., 2007). The complex tight junctions and multiple cell layer structure make the brain virtually inaccessible for polar molecules, unless they are transported by specific pathways at the barrier. Therefore, for most solutes and macromolecules, their permeability across the BBB is dependent upon their lipophilicity and size. Many small water soluble nutrients and macromolecules needed for proper CNS function can cross the barrier through specific carrier mechanisms or facilitated diffusion. Substances that can cross the BBB from the blood then enter the brain interstitial fluid.

Unlike the BBB, the BCB is located in the choroid plexus, a highly vascularized and polarized tissue in the roof of each of the four brain ventricles. The structural basis of the BCB is the tight junctions in the choroidal epithelial cells. Under a microscope, the choroid plexus is composed of three cellular layers: (i) the apical epithelial cells facing the CSF, (ii) the underlying supporting connective tissue, and (iii) the inner layer of endothelial cells with immediate contact with the blood. The surface area of the BCB is about 50% that of the BBB or within the same order of magnitude as the BBB (Keep and Jones, 1990; Speake and Brown 2004). Given that the endothelial cells of the choroid plexus are functionally leaky, the passage of substances across this barrier is essentially controlled by the tight junctions in the apical layer of epithelial cells. The BCB allows only select materials to gain access to the CSF, while the fluxes of most water soluble substances, proteins, ions, and macromolecules are impeded from the blood to the CSF. The primary function of the BCB is to actively produce and secrete the CSF along with critical molecules such as transthyretin and transferrin into the brain. Beyond restriction of the access of substances from the blood to the CSF, the BCB is known to remove substances from the CSF to the blood (Zheng et al., 2003).

Both barriers selectively transport essential nutrients, metals, and drug molecules into the CNS. During the last few decades, the role of these brain barrier systems in controlling CNS metal homeostasis has received substantial attention (Zheng et al., 2003; Yokel, 2006). With regard to the high selectivity of metal/chemical accumulation in particular brain regions, the concept of a brain regional barrier has been brought up and discussed elsewhere (Zheng, 2001).

3. Iron Transport by Brain Barriers

3.1. Systemic Fe homeostasis and regulation

The human body has an average of 4–5 grams of Fe, about 65% of which is in the form of hemoglobin and 4% in the form of myoglobin. Another 15 to 30% is stored mainly in the reticuloendothelial system and liver parenchymal cells, primarily in the form of ferritin. The metabolism and systemic balance of Fe in the body is regulated by several macromolecules summarized in Table 1.

Once Fe enters the circulation from the small intestine, it binds to the β-globulin apotransferrin, to form transferrin. Transferrin carries Fe in the blood and serves as the major vehicle for Fe transport in the body. Upon arriving at the target cells, transferrin binds selectively with transferrin receptors (TfR) on the outer surface of the cell membrane. Through endocytosis, transferrin carries Fe into the cell, where the Fe is released, and the free Fe is either utilized in metabolic processes, or conjugated with a large molecular weight protein apoferritin (460,000 Dalton) to form ferritin, which functions as the cellular storage site for Fe.

3.2. Fe transport at the BBB

Under normal physiological conditions, the brain stringently regulates Fe balance by possibly three well-coordinated systems: (a) the influx of Fe into brain either via TfR-mediated transport or the non-TfR mediated transport at brain barriers, (b) the storage of Fe in which the cellular sequestration is largely dependent upon availability of ferritin, and (c) the efflux of Fe whose rate is controlled by bulk CSF flow and/or by the removal mechanism in the BCB back to the blood circulation (Bradbury 1997; Connor and Benkovic, 1992; Jefferies et al., 1984; Wang et al., 2008, 2009).

It is commonly accepted that Tf-bound Fe is the primary species transported into the brain. The Tf-Fe can be taken up by endocytosis into cerebral capillary endothelia where the molecules subsequently dissociate. Apotransferrin is then recycled to the blood compartment, where the released iron is transported across the abluminal membrane of the barriers into the cerebral compartment. The question as to how exactly the intracellular Fe is exported out of the abluminal side of endothelia remains controversial, as the involvement of ferroportin (FPN) in Fe cellular trafficking in the endothelia is still in dispute (Moos 2007; Wu et al., 2004). Nonetheless, the Fe species that are transported out of abluminal membrane can bind to brain Tf derived discretely from oligodendrocytes and choroid plexus epithelia, and become available for neurons or neuroglia expressing transferrin receptors (TfR) (Beard and Connor, 2003). Hence, transferrin, transferrin receptor, and ferritin are quintessential to the regulation of Fe in the CNS.

TfR-mediated Fe transport by the BBB may partially explain the unique distribution pattern of Fe in brain tissues. In the normal brain, Fe concentrations are high in the striatum and hippocampus, and low in the cortex and brain stem (Morris et al., 1992; Sugawara et al., 1992). Deane et al. (2004) used a receptor-binding assay to determine the kinetic parameters of Tf binding to TfR on brain capillary endothelium collected from selected brain regions. Their data show that the maximal density (B_{max}) of TfR in cerebral endothelia is about 3–7 fold higher in the striatum and hippocampus than in the cortex. Thus, the observation supports the theory that unevenly distributed TfR in cerebral capillaries may underscore the uneven distribution of Fe throughout brain regions. In the same study, the authors also observed that the rates of ^{59}Fe-Tf regional uptake were about 2–3 fold higher in the striatum and hippocampus than in the cortex, which correlates well with the results of TfR regional density in the brain endothelium.

While TfR-mediated Fe transport at the BBB is well established, unbound, free Fe may constitute yet another significant pathway for Fe to gain access to the brain. Ueda et al. (1993) used anti-TfR antibodies to create the maximal inhibition of TfR-mediated Fe transport. Yet they were still able to find that about 35–65% of non-TfR bound Fe ions were transported into the brain. In the transgenic hypotransferrinemic mice whose transferrin levels were less than 1% of the normal level, a significant brain Fe uptake was also observed after intravenous injection of $^{59}FeCl_3$ (Malecki et al. 1999). Takeda et al. (2002) also found the evidence of non-Tf mediated brain Fe uptake, when they intravenously injected $^{59}FeCl_3$ into a Tf-saturated mouse model and discovered that ^{59}Fe species were taken up by the brain in appreciable amounts. A more recent study by Deane et al., (2004) compared the unidirectional uptake rates (K_{in}) by tissues between free and Tf-bound Fe species. Neither the brain capillaries nor the capillary-depleted brain parenchyma showed any significant differences in accumulation of ^{59}Fe, no matter how the Fe, either as free or Tf-bound species, was administered. Physiologically, less than 1% Fe in blood circulation is unbound. Thus, the free Fe in the local brain microcirculation must stem from the dissociation from its carrier Tf. Indeed, there is evidence in the literature suggesting an increased Fe release from Tf upon binding of Tf-Fe complexes to TfR (Bali et al. 1991; Sipe and Murphy 1991). Assumedly, this would increase free Fe within the local brain microcirculation, and the

unbound free Fe could then be taken up by divalent metal transport-1 (DMT1) or other metal transporters in the BBB. It is also possible that the free Fe in local brain parenchyma may be derived from the cellular release of Fe.

DMT1, also known as divalent cation transporter 1 (DCT1) or Nramp2 (natural resistance associated macrophage protein 2), is a proton driven transporter, transporting one proton and one atom of ferrous Fe(II) in the same direction. In addition, DMT1 nonselectively transports multiple divalent metals including manganese (Mn), copper (Cu), cobalt (Co), zinc (Zn), cadmium (Cd) and lead (Pb) (Gunshin et al., 1997). DMT1 is expressed in most organs and has been identified in duodenal epithelia, erythrocytes, renal epithelial cells, cardiomyocytes and human placenta epithelia (Ferguson et al., 2001; Georgieff et al., 2000; Ke et al., 2003; Roth et al., 2000). In the brain, DMT1 has been shown to exist in neurons (Burdo et al., 2001). The question as to whether the DMT1 is expressed in the BBB has not yet been fully addressed. Siddapappa et al. (2002) reported the presence of DMT1 in brain capillary endothelial cells. Other researchers have also suggested that a DMT1-mediated Fe dysregulation at the BBB after manganese (Mn) exposure may contribute to Mn-induced neurotoxicity (Roth and Garrick, 2003). Moreover, a high expression of DMT1in nuclei of basal ganglia, particularly the caudate nucleus, putamen, and substantia nigra, has been suggested to account for the high levels of Fe in these regions (Huang et al., 2004).

However, Moos et al. (2006) utilizing Belgrade rats with a mutation in DMT1 suggested that DMT1 is not essential for Fe transport via the BBB. The same group of investigators reported that when a panel of antibodies raised against different regions of the DMT1 molecule, such as the conserved trans-membrane region and the variable C-terminal region with or without an iron responsive element (+IRE vs. −IRE), were used for detecting DMT1, they were unable to identify DMT1 in brain endothelial cells (Moos and Morgan 2004). These data seem to suggest that the brain capillary endothelial cells may express DMT1 in a relatively low level. Thus, how free Fe is transported, aside from DMT1, across the BBB, remains a highly interesting question for further exploration.

3.3. Fe transport at the BCB

The presence of Fe transporters (TfR, DMT1 and FPN) and Fe storage and regulatory proteins (Ft, IRPs, Hepcidin) in the choroid plexus has been demonstrated in the literature (Table 1). There are two major DMT1 mRNA transcripts, one of which contains an iron responsive element (IRE) with a stem-loop structure in the 3′-untranslated regions (UTR) that is available for interaction with cellular proteins (Addess et al., 1997; Eisenstein and Ross, 2003; Konarska and Sharp, 1986; Rouault, 2001). The stem-loop structure of IRE has also been identified and characterized in mRNAs encoding TfR. Literature data have established that the IRE structure in TfR mRNA can uniquely bind to iron regulatory protein-1 (IRP1), whose active center contains a [4Fe-4S] cluster (Fig. 2) (Zheng et al., 1998). In the Fe-deficient condition, IRP1 binds with a high affinity to IRE containing TfR mRNAs, stabilizes TfR expression and increases cellular levels of TfR (Andrews, 1999; Klausner et al., 1993; Li et al., 2005). Mn has been shown to replace one Fe in the [4Fe-4S] in IRP1, resulting in the inhibition of the enzyme's catalytic function consequently increasing its binding activity to IRE-containing TfR mRNA. This event leads to an increased expression of TfR and cellular overload of Fe in the choroid plexus (Li et al., 2005, 2006; Zheng et al., 1998). Wang et al. (2006) utilized the electrophoretic mobility shift assay to determine the binding activity between cellular IRP1 and DMT1 mRNA whose sequence contains a stem loop similar to TfR mRNA at 3′-UTR (Fig. 2). The study demonstrated significant binding between IRP1 and the DMT1 stem-loop mRNAs in choroidal epithelial cells. This observation is consistent with studies conducted by Gunshin et al. (2001), who conclude that DMT1 mRNA is regulated through stabilization by the IRE-IRP system, in an analogy to TfR regulation.

The pattern of subcellular distribution of TfR, DMT1, FPN in choroid plexus tissue is quite distinct with regard to the apical vs. abluminal epithelial structure (Wang et al., 2008a). DMT1 is mainly distributed in a polarized pattern towards the apical plasma membrane, while FPN is less polarized and more diffusely distributed in the cytoplasm. Unlike DMT1 and FPN, TfR is mainly localized around nuclei in the form of clusters, which appears to suggest a ready availability of TfR to either the apical or abluminal membrane. Noticeably, none of these transporters exists in the nucleus. This pattern may indicate, as discussed below, the different roles of the proteins in transporting Fe between blood and CSF compartments.

When blood-borne Fe enters the capillaries of the choroid plexus network, the fenestrated plexus endothelia allow Fe molecules, either TfR-bound or free, to come into contact with the abluminal membrane of the choroidal epithelia. Similar to the event at the BBB, Tf-bound Fe binds with a high affinity to the TfR; the complex then enters the cytoplasm by receptor-mediated endocytosis. The dissociation of Fe from transferrin occurs in the low-pH endosomes, where FPN may export Fe from the cytosol to the CSF. TfR proteins are known to be available to both sides of the choroidal cell surface membrane (i.e., blood vs. CSF), whereas DMT1 is predominantly present on the apical membrane that faces the CSF. Such a polarized DMT1 distribution may determine the direction of Fe flux across the BCB. Indeed, in a two-chamber Transwell culture model with primary choroidal epithelial cells, free Fe was found to be transported by the choroid plexus favorably from the CSF toward the blood (Wang et al., 2008b). The efflux of Fe from the CSF to the blood, as corrected by a leakage marker, was predominant relative to Fe influx. The use of siRNA to knock down DMT1 significantly reduced cellular Fe uptake. Since free Fe can exist on the cell surface of both sides of the choroidal membrane, it makes sense that in addition to TfR-mediated transport, DMT1 may selectively pick up free Fe from the CSF and transport it back to the blood. Thus, DMT1-mediated Fe efflux in the BCB may constitute a clearance mechanism upon which the Fe concentration in brain extracellular fluid is maintained at a relatively stable level. Considering the steep concentration gradient of Fe between the blood (~1.150 mg/L) and CSF (0.02 mg/L) (Zheng et al., 1999), the BCB-mediated efflux of Fe must be an energy-dependent, active transport process.

One could argue that the large volume and constant production of CSF from the choroid plexus may act as a “sink” to remove excess Fe molecules diffused from the interstitial fluid, and thus there is no need for a BCB clearance pathway. It should be pointed out that the live choroid plexus tissue is loosely floating in the CSF and fills the entire space of brain ventricles. The apical microvilli and multiple basolateral infoldings confer the tissue with a surface area that is in the same order of magnitude as that of the BBB (Keep and Jones, 1990; Zheng, 2005). Thus materials freshly entering the CSF from the interstitial fluid will initially come into contact with the choroidal microvilli in brain ventricles. There are ample examples of removal of unwanted materials including beta-amyloid proteins by the choroid plexus (Crossgrove et al., 2003; Redzic, 2011; Zheng et al., 2003). Moreover, the rich blood flow to the choroid plexus, which is about 4–6 fold faster than that of other brain regions, may assist the BCB in removing Fe from the CSF.

3.4. Overall Fe transport in brain

In summary, the observations from this laboratory (Deane et al., 2004) have established that the rate of Fe uptake in the CNS occurs in the following order

brain capillaries > choroid plexus > brain parenchyma > CSF

Based on this rate-uptake order, one can postulate that Fe ions in the blood circulation are primarily transported into the brain parenchyma by the BBB. The correlation between the TfR density and Fe concentrations in various brain regions further suggests that a TfR-

mediated transport mechanism at the BBB is primarily responsible for regulation of the rate of Fe entry to the brain parenchyma. Once inside the brain interstitial fluid, Fe is carried by brain Tf for neuronal activities. The excess Fe in neurons and neuroglia can be spilled into the brain interstitial fluid; from there and through bulk flow, Fe, either as a Tf-bound or free species, is released into the CSF in the brain ventricles. The apical microvilli of choroidal epithelia then capture the free Fe via DMT1 or Tf-bound Fe via TfR, and transport the Fe into the blood. Overall, it seems likely that Fe enters the brain through the BBB and exits the brain via the BCB. This mechanism is illustrated in Fig. 3.

4. Copper Transport by Brain Barriers

4.1. Systemic Cu homeostasis and regulation

The body of a healthy adult possesses roughly 110 mg of Cu, primarily distributed in the liver (10 mg), brain (8.8 mg), blood (6 mg), bone (46 mg), and muscle (26 mg) (Linder, 1991; Linder et al., 1996). The majority of Cu ions are absorbed in the small intestine and delivered to the liver and kidneys. In the liver, most of the Cu ions are bound to ceruloplasmin and released into the blood. In serum, nearly 65–90% of Cu is tightly bound to ceruloplasmin, with the rest being loosely bound with albumin, transcuprein, and amino acids (Bradbury, 1992; Choi et al., 2009).

Maintenance of cellular Cu transport and homeostasis requires various membrane Cu transporters and a subset of intracellular proteins known as Cu chaperones which deliver Cu to specific intracellular targets. The membrane-associated Cu transporters include copper transporter-1 (CTR1), DMT1, and Cu exporter ATPases (ATP7A and ATP7B). The chaperones include antioxidant protein-1 (ATOX1), cytochrome oxidase enzyme complex (COX17), and Cu chaperone for SOD (CCS) (Harris et al., 2001).

Once inside the cell, Cu has four possible fates: (i) it can enter the Cu-metallothionein storage pool; (ii) it can be transported to the mitochondria for incorporation into cytochrome c oxidase; (iii) it can be incorporated into cytoplasmic Cu/Zn SOD; and (iv) it can be transported to a P-type ATPase in the trans-Golgi network for transport out of the cell (Bressler et al., 2007; Choi et al., 2009). Recent research has demonstrated that many of these cellular Cu transporters responsible for cellular Cu trafficking are present at the BBB and BCB (Choi et al., 2009).

4.2. Species of Cu transported by brain barriers

Since the majority of Cu ions in the blood are bound to plasma proteins, it is tempting to postulate that Cu may be transported in a metal-protein complex form similar to the Tf-bound Fe. Indeed, some earlier studies have demonstrated the presence of unique membrane receptors for ceruloplasmin, which supports the role of ceruloplasmin in distributing and metabolizing Cu (Hsieh and Frieden, 1975; Campbell et al., 1981; Stevens et al., 1984). However, Meyer et al. (2001) conducted experiments in a transgenic mouse model with a low level of ceruloplasmin and found that systemic Cu kinetics with regard to gastrointestinal absorption, hepatic uptake, or biliary excretion do not differ significantly between aceruloplasminemic mice and wild-type mice. Thus, the authors concluded that ceruloplasmin is not essential for Cu absorption and metabolism.

To seek direct experimental proof, Choi et al. (2009) prepared three Cu species, i.e., free ^{64}Cu, ^{64}Cu-albumin, and ^{64}Cu-ceruloplasmin, and used an in situ brain perfusion technique to infuse these Cu species directly into the rat brain in order to determine the unidirectional Cu uptake rate. The procedure was followed by a capillary depletion method to separate the brain capillary fraction from the brain parenchyma so that the Cu retained in the capillary can be distinguished from that present in the brain parenchyma. The time

course of these studies indicated a linear Cu uptake into the choroid plexus, CSF, brain capillaries and capillary-depleted parenchyma. The data revealed that the volume of distribution (V_d) of free Cu in the choroid plexus is about 50 times greater than that of albumin-bound Cu, and about 1000 times greater than that of ceruloplasmin-bound Cu. A similar pattern of Cu transport with these three species exists in brain capillaries, parenchyma, and CSF.

Albumin-bound Cu is unlikely the species that transports Cu into the brain. Vargas et al. (1994) have shown that there is no significant difference in Cu distribution and metabolism between albumin-null and wild-type animals. Since Cu is loosely bound to albumin (Pietrangelo et al., 1992), it is possible that Cu ions may be released from albumin-bound moiety at the brain barriers and that these free Cu ions may subsequently be transported into the brain. Collectively, these studies suggest that the free, unbound Cu species in the systemic circulation or at the site of brain transport are the major species entering the brain barriers and being transported into the brain parenchyma.

4.3. Transporters responsible for Cu uptake at brain barriers

Little work has been done on the fluxes of Cu across the BBB or BCB. The Cu concentration in the CSF has been estimated to be 0.3–0.5 μM or 22.3 ± 2.2 μg/L. Serum Cu concentration (1129 ± 124 μg/L) is considerably higher (Forte et al., 2004; Gellein et al., 2007; Nischwitz et al., 2008). The substantial difference between these two fluid compartments suggests that the brain barriers effectively regulate Cu in the CNS. Table 2 summarizes the major proteins involving Cu regulation.

Copper transporter 1 (CTR1) is a plasma membrane protein with 3 transmembrane domains that form a homotrimeric pore essential for Cu uptake, as Cu(I) in all cells. Cu (II) is thought to be reduced to Cu (I) by various Steap proteins on the cell surface before uptake by CTR1. Cu uptake by CTR1 is (i) energy dependent, (ii) stimulated by acidic extracellular pH and high K+ concentrations, and (iii) time dependent and saturable (Gaggelli et al. 2006). Evidence suggests that CTR1 may be post-translationally regulated by intracellular Cu availability. Elevated intracellular Cu levels stimulate the rapid endocytosis and degradation of CTR1 from the cell membrane, resulting in attenuated Cu uptake and thus preventing the cellular overload of this redox active metal (Gagelli et al. 2006). CTR1 has been shown to be present in the brain capillary endothelial cells of the BBB, choroid plexus of the BCB (Fig. 4A), as well as the brain parenchyma (Choi et al., 2009; Kuo et al. 2006). Recent data from this laboratory show that chronic exposure to Mn in rats leads to the subcellular relocation of CTR1, rendering a highly concentrated CTR1 in the apical brush boarder of the choroidal epithelia facing the CSF (Fig. 4B). The exact implication of this intracellular trafficking of CTR1 at the BCB in response to Mn exposure remains to be determined.

Uptake of Cu by the brain barriers may be also mediated by DMT1. DMT1 has been shown to be partially responsible for Cu transport in Caco-2 cells, and that Fe and Cu can competitively inhibit each other's transport by DMT1 (Arredondo et al., 2003). In addition, it is known that both Fe and Cu levels regulate the translational expression of DMT1 via the binding and unbinding of IRP1 to the stem-loop structure of mRNA encoding DMT1 (Collins et al., 2006; Han et al., 2002). Additionally, experimental evidence has shown that a Fe-deficient condition can increase intestinal tissue Cu levels and enhance the expression of both DMT1 and ATP7A in the intestine (Collins et al., 2006). Whether similar regulatory events also occur to DMT1 and ATP7A at the brain barriers remain to be further illustrated. It is possible that altered ATP7A expression and function could affect Cu homeostasis in the CNS, resulting in potential damage to brain function. It should be noticed that DMT1 expression in the BBB seems to be less abundant than in the BCB, as discussed in Section 3.2. Thus, at the BBB, CTR1 may be a predominant transporter in regulating Cu uptake by

the endothelial cells. Current research has demonstrated that CTR1 immunostaining is intense in BBB endothelial cells and some researchers have proposed that CTR1 may be located on the luminal side of these barrier cells, although this theory has yet to be confirmed (Kuo et al., 2006; Kaler, et al. 2011).

4.4. Intracellular Cu trafficking and storage at brain barriers

Cu chaperones are part of a larger family of metallochaperones, structurally adapted to bind Cu, and function to recognize recipients and conduct exchange reactions with target proteins. Currently at least three chaperones are known to perform the intracellular Cu trafficking in eukaryotes. ATOX1 (formerly HAH1) is responsible for transporting Cu to the Cu-ATPases residing in the trans-Golgi network. ATOX1 binds Cu(I) and associates with ATP7A and/or ATP7B to deliver Cu(I) to nascent cuproenzymes or efflux it from the cell. ATOX1 has been found to be expressed in the choroid plexus and brain capillary endothelial cells (Nishihara et al., 1998).

COX17 functions to transfer cytosolic Cu to the mitochondria. While it has been reported that this protein is present in the neurons of the brain, it has yet to be confirmed that it is located in either brain capillary endothelial cells or choroid plexus (Kaler et al., 2011).

CCS is the chaperone required to insert copper into Cu-Zn SOD in mammals. The mRNA and protein levels of CCS are sensitive to the intracellular Cu concentration (Bertinato et al, 2003). Cu deficiency in mice resulted in an increase in CCS levels in the cerebellum, but remained unchanged in the choroid plexus (Gybina et al., 2006). It is unclear how ATOX1, COX17 and CCS may respond to changes in Cu levels in the systemic circulation as well as in the CSF. It is also currently unknown how the levels of expression of these Cu chaperones may affect the directional trafficking of Cu atoms within the BBB and BCB.

Metallothionein (MT) is comprised of a group of metal binding, low molecular weight proteins with high cysteine content. MT has been shown to exist in the BBB and BCB (Nishimura et al., 1992). A recent study has demonstrated that the Cu-MT binding constant is much larger ($>10^{19}$ M^{-1} at pH 7.4) relative to zinc (Zn) (3.2×10^{13} M^{-1}) (Ba et al., 2009). As a result of the higher Cu-MT binding affinity relative to Zn, MT will exchange Zn for Cu when excess Cu is present as a defense strategy against the more toxic Cu ions. MT binding of Cu at the brain barriers likely plays a role in regulating intracellular Cu storage. In coordination with CTR1 and/or DMT1, the intracellular free Cu, either taken up by these transporters or released from MT, may meet the demand of brain's functional needs for transport. At this stage, the precise mechanism of this coordination is unknown.

4.5. Transporters responsible for Cu export at brain barriers

Two intracellular proteins, i.e., ATP7A and ATP7B belonging to a subclass of ATPases, are responsible for ATP-dependent transport of Cu across the brain barriers. Both proteins possess eight transmembrane domains and contain six Met-X-Cys-X-X-Cys Cu binding motifs at the N-terminus (Lutsenko et al., 2007). While both ATP7A and ATP7B are present in the brain capillary endothelial cells as well as in the choroidal epithelial cells (Choi et al., 2009; Nishihara et al., 1998), their relative expression levels in the BBB and BCB seem to be different. The level of ATP7A is roughly 3–4 fold higher in the BCB relative to the BBB. In contrast, the expression of ATP7B is about 4–5 fold higher in the BBB compared to the BCB (Choi et al., 2009). Under a microscope, ATP7A molecules in the choroidal epithelial cells are evenly distributed in the cytosol (Fig. 4C). However, the distribution pattern of ATP7A and ATP7B in the BBB are unknown. More detailed experiments are needed to qualify the protein expression of ATP7A and ATP7B at both barriers.

Functionally, both ATPases are localized to the trans-Golgi network, where they are utilized in the Cu secretory pathway, delivering Cu for incorporation into cuproenzymes. When intracellular Cu levels rise, both ATP7A and ATP7B are known to translocate from the Golgi network and carry Cu to the cellular membrane for efflux from the cell. This function is not only necessary for Cu trans-barrier transport, but also important for preventing excess Cu accumulation inside the barrier cells. The exact functional difference between ATP7A in the BCB and ATP7B in the BBB remains unknown.

A dysfunction in these Cu ATPases can have a direct impact on brain Cu levels. For example, a shutdown of ATP7A gene expression can result in a Cu accumulation in brain capillaries of macular mutant mice (Yoshimura et al., 1995) with an ensuing deficiency of Cu to the brain, suggesting that this protein plays a role in BBB Cu transport. Transcriptional control has been reported for both ATP7A and ATP7B in the CNS. ATP7A expression is triggered by retinoic acid receptor β in neuroblastoma cells (Bohlken et al., 2009). The pineal gland specific form of ATP7B appears to be under the control of cone rod homeobox (CRX), a pineal/retina specific nuclear protein (Li et al., 1998). In addition to transcriptional control, both ATP7A and ATP7B appear to be subject to alternative splicing (Collins et al, 2009; Loudinos et al., 2002). Aside from the known genetic disorders Menkes' disease and Wilson's disease (which will be discussed in Section 5), the localization, regulation and directional trafficking of both these Cu exporters at the brain barriers, and their respective roles in CNS Cu homeostasis, remain largely unknown.

4.6. Relative role of BBB and BCB in Cu transport

The mechanism as to how Cu is transported into and out of the brain at both the BBB and BCB is not fully understood. Under normal physiological conditions, the brain barriers are impermeable to Cu. Movement of Cu across the brain barriers between two fluid compartments requires specific Cu transport systems. Only under certain pathological conditions, in which brain barrier permeability is compromised, may Cu enter the CSF through passive diffusion. This is seen, for example, in patients with meningitis. Cu transport at the brain barriers is achieved by a coordinate series of interactions between passive and active transport proteins.

Available data indicate that Cu levels may be regulated in a cell specific manner at the levels involving transcriptional, translational, and posttranslational control. Current models suggest that Cu is taken up from the blood by CTR1 and transported across the BBB via ATP7A (Lee et al., 2001). Additional data suggests that Cu is taken up from the CSF via CTR1 enriched on the apical surface of the choroid plexus (Kuo et al., 2006).

The relative contribution of the BBB and BCB to CNS Cu regulation depends on several factors, i.e., the direction to which the barrier guides Cu flow, the rate with which the barrier delivers Cu molecules, and the surface area through which the barrier transmits the volume. Data by Choi et al. (2009) and Monnot et al. (2011) suggest that among the cerebral capillary, choroid plexus, brain parenchyma and CSF, the choroid plexus exhibits the highest capacity in acquiring Cu from the blood circulation irrespective to what Cu species is used. Logically, such a large capacity in Cu uptake by the BCB should have warranted a high influx of Cu into the CSF compartment since most of the CSF is produced by the choroid plexus. It is therefore surprising to see that the Cu uptake rate by the CSF is nearly 1200 fold slower than that in the BCB. This may bespeak to the fact that the choroid plexus has the ability to sequester Cu and thus tightly regulate the movement of Cu from the blood into the CSF. In brain parenchyma, however, the Cu uptake rate is much faster than the uptake rate to the CSF, indicating that Cu is transported at a greater rate to the brain parenchyma than to the CSF. Thus, while Cu uptake by the BBB is slower (about 3.2 fold less) than that of the BCB, the acquired Cu ions in cerebral capillaries appear to be more

efficiently transported to the parenchyma than Cu in the choroid plexus to the CSF. Since there is no apparent barrier between the CSF and interstitial fluid, the Cu in the CSF may be derived from the Cu in the bulk flow of the interstitial fluid spilled by neurons and glial cells. Considering the larger surface area and more direct contact with neuronal structure of the BBB than the BCB, the BBB appears to be a more important route than the BCB in the transport of Cu into the brain parenchyma.

To address the directional transport of Cu by the BCB, Monnot et al. (2011) used a two-chamber Transwell culture system with the primary choroidal epithelial cells to compare the rate of Cu transport between the blood and CSF. The data demonstrated that the rate of Cu transport from the CSF to the blood is greater than that from the blood to the CSF. An additional in situ ventriculo-cisternal perfusion study also supports the role of the choroid plexus in the uptake of Cu from the CSF (Monnot et al., 2011). At present, the question as to whether or not the BBB transports Cu from the brain interstitial fluid back to the blood is unanswered.

Collectively, it is reasonable to postulate that Cu in the blood stream enters the brain parenchyma mainly through the BBB. The free, unbound Cu ions is likely taken up by CTR1 in cerebral endothelia, from there ATP7B along with Cu chaperones may translocate Cu to the abluminal membrane and release it into brain interstitial fluid for neuronal activities. Subsequently, Cu may flow from the brain parenchyma to the CSF in brain ventricles, where it can be taken up by CTR1 and DMT1 present in choroidal epithelial microvilli. A small fraction of these Cu ions may be removed by the CSF bulk flow to arachnoidal granulation for drainage. In a similar fashion to Fe transport in the BCB, the choroidal epithelial cells transport Cu against the concentration gradient to the blood. Thus, the BBB appears to determine the influx of Cu to the brain whereas the BCB functions as the regulatory site to maintain Cu homeostasis in brain extracellular fluid.

5. Perspectives: Iron or Copper Dysregulation in Neurodegenerative Diseases

5.1. Disorders due to genetically abnormal metal transport by brain barriers

Increasing evidence suggests that both Fe and Cu play a critical role in many neurodegenerative diseases (Gotz et al., 2004; Kell, 2010; Zecca et al., 2004). Conditions such as neuroferritinopathy and Friedreich ataxia are associated with mutations in genes encoding proteins that are involved in Fe metabolism. Fe has been found to accumulate in particular brain regions and its level increases with the severity of neuropathological changes in idiopathic Parkinson’s disease, presumably due to an increased transport through the blood-brain barrier in the late stages of Parkinsonism. High levels of reactive Fe can then facilitate the formation of reactive intermediates, leading to neuronal damage (Kell, 2010).

The genetic disorders Menkes disease and Wilson’s disease originate from improper Cu homeostasis in the CNS. Menkes disease is a neurologic disorder caused by mutations in an X-linked gene, ATP7A. Patients with this disease display significant neurological and developmental impairment due to disrupted Cu delivery to the brain, resulting in brain Cu deficiency, leading to neuronal degeneration (Lutsenko et al., 2003; Liu et al., 2005). These individuals also have a variety of other symptoms as a result of decreased function of Cu-dependent enzymes including connective tissue abnormalities, and a lack of pigmentation.

Mutations in the gene encoding the Cu-ATPase ATP7B results in a severe metabolic Cu overload disorder known as Wilson’s disease. Inactivation of this transporter is associated with Cu accumulation in the liver and brain, resulting in a variety of hepatic and

neurological abnormalities. These include liver dysfunction or failure, movement disorders, and psychiatric disturbances (Lutsenko et al., 2007; Ferenci, 2004; Gitlin, 2003).

5.2. Neurological diseases and disorders due to altered brain homeostasis of Fe and Cu

Amyotrophic lateral sclerosis (ALS) is a neuromuscular disorder characterized by gain of function mutations in the cytosolic Cu enzyme Cu/Zn-SOD. Some evidence suggests a pathogenic role for Cu in this process primarily through enhanced free radical generation. In the brains of ALS patients, the epithelial cells of the choroid plexus show a high expression of both Cu/Zn-SOD and Mn-SOD (Uchino et al., 1994). Interestingly in a transgenic mouse model of ALS, administration of polyamine-modified catalase, which increases the permeability of the BBB, delays the onset of symptoms and increases animal survival, suggesting a possible role of the brain barriers in ALS (Poduslo et al., 2000).

Prion disease is a neurodegenerative disorder that results from the misfolding of endogenously expressed cellular prion protein. The prion protein is a cell surface glyco-protein expressed by neurons. The protein has a strong binding capacity to Cu; thus, its expression on the cell surface has been suggested to regulate Cu uptake into cells (Brown, 2001; Pauly and Harris, 1998). An altered Cu metabolism may contribute to prion protein misfolding. Recent studies have shown that exposure to Mn up-regulates prion protein expression (Choi et al., 2010; Martin et al., 2011). Although the exact mechanism remains unknown, it is possible that Mn may interact with cellular Cu leading to prion disorders.

An imbalance in regulating extracellular and intracellular Cu transport in the CNS has been suggested to play a role in the etiology of other neurodegenerative diseases. Plaques isolated from brains of patients with Alzheimer's disease display high levels of Cu (Phinney et al., 2003). In addition, trace amounts of Cu in water have been shown to induce β-amyloid plaques in a rabbit model of Alzheimer's disease (Sparks et al., 2003).

Cu, Fe and other metals such as Zn are known to be highly reactive. The cuprous form of Cu and ferrous form of Fe catalyze the generation of the powerful oxidizing agent hydroxide from hydrogen peroxide. It has been suggested that the oxidative damage found in brains from patients with Alzheimer's disease and Parkinson's disease is generated by metals such as Fe and Cu (Barnham et al., 2004; Kell, 2010). In addition, hydrogen peroxide can be generated by adding Cu to β-amyloid (Opazo et al., 2002). In order to prevent this type of oxidative damage, Fe, Cu and other metals are under strict regulation by extracellular and intracellular transporters, and binding proteins. A breakdown in the regulatory mechanisms of Fe and Cu homeostasis, particularly at either of the brain barriers, may, in part, underlie the development of neurodegenerative diseases.

Recent studies have also indicated that elevated free Cu levels in serum are associated with cognitive decline (Salustri et al., 2010). The investigators reported a significant inverse correlation of the serum levels of free Cu with both the Mini Mental State Examination and attention-related neuropsychological test scores. This is certainly an interesting finding; if the brain barriers functioned properly, one would expect that brain Cu homeostasis can be maintained adequately regardless of serum Cu status, protecting the brain from Cu-induced pathogenesis.

It is also interesting to notice that the concentration of Fe in the brain increases with age and this increase becomes even more prevalent in the brains of subjects with neurodegenerative diseases such as Parkinson's disease (Dexter et al. 1987; Sofic et al. 1988). Whether this is due to increased uptake at the BBB, non-specific paracellular leakage, or decreased clearance by the BCB will become an interesting research subject for further investigation.

5.3. Environmental exposure and dysfunctional Fe and Cu transport by brain barriers

Exposure to environmental toxicants, such as rotenone, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and Mn can induce Parkinson's type syndromes in humans and animals. The neurotoxicities of these toxicants have been associated with altered Fe status at local and/or systemic levels (Betarbet et al., 2000; Crossgrove and Zheng, 2004; Zecca et al., 2004; Zheng et al., 1999). In addition, a general increase in Fe concentrations in the substantia nigra has been observed in animals following treatment with 6-hydroxydopamine (6-OHDA), ibotenic acid infusion (to destroy GABAergic neurons), and direct intranigral Fe infusion (He et al., 1996; Sastry and Arendash, 1995). LaVaute et al. (2001) have further reported that the knockout of the IRP-2 gene in mice results in dysregulation of Fe metabolism in the brain and ensuing neurodegenerative disorders. The mice are normal at birth and gradually accumulate Fe in the CNS developing neuropathological symptoms.

Occupational exposure to Mn has also been shown to lead to symptoms resembling Parkinson's disease (Crossgrove and Zheng, 2004; Jiang et al., 2007; Zheng et al., 2011). Animal studies suggest that Mn exposure leads to low blood Fe levels and elevated CSF Fe levels, presenting a compartmental shift of Fe from the blood to the CSF (Li et al., 2005, 2006; Zheng et al., 1999). By using a confocal microscopic technique, results from this laboratory have indicated that Mn exposure translocates FPN and TfR in the choroidal epithelia toward the apical membrane of the choroidal epithelia that face the CSF, while DMT1 distribution in the cells remain unchanged. Fe efflux from the CSF to the blood is also reduced. Thus, the enhanced TfR-mediated influx of Fe from the blood to the choroid plexus and the increased export of Fe to the CSF by FPN may underlie the elevated Fe concentration in the CSF (Li et al, 2006; Wang et al., 2008a,b). Further experiments reveal that Mn can replace the 4th loose Fe in the [4Fe-4S] active center of IRP1. In this state IRP1 shows a high affinity to the stem-loops in the 3′-UTR of TfR and DMT1mRNAs and in 5′-UTR of FPN mRNA (Li et al., 2005, 2006; Zheng et al., 1998). Since the heterogeneous nuclear RNA assay and nuclear run-off assay for nascent RNA did not find any significant changes of TfR and DMT1 following in vivo Mn exposure, it seems likely that Mn treatment does not affect the rate of gene transcription and RNA processing of DMT1 and TfR. Thus, Mn toxicity on brain transport of Fe is due to its effect on DMT1 and MTP1 expression at the translational but not transcriptional level.

5.4. Metal-metal interaction at brain barriers

Cu itself is not a barrier destroyer; but the presence of Cu may alter the transport of Fe. This was seen in rats fed a Cu-enriched diet, where the influx of Fe into the brain was significantly decreased compared to that of rats fed with the control diet (Crowe and Morgan, 1996). Exposure to other metals may interfere with Cu transport by the brain barriers. Qian et al. (1999) demonstrated that Pb accumulation in C6 rat glioma cells altered the membrane transport properties for Cu, leading to an increased uptake and a decreased efflux of Cu.

Available experimental evidence also suggests an intimate relationship between the metabolic roles of Cu and Fe in humans (Fairweather-Tait et al., 2004; Gambling et al., 2004, Sharp et al., 2004). Recent studies by Monnot et al. (2011) have demonstrated that systemic Fe levels may alter the transport of Cu across the brain barriers. In a Fe-deficient rat model, a significant increase (+55%) of Cu levels in the CSF, brain parenchyma and the choroid plexus was observed, while no effect was seen on CSF Fe levels. Experiments to examine the rate of Cu transport via an in situ brain perfusion technique demonstrated that the rate of Cu transport into the brain parenchyma was significantly faster in Fe-deficient rats (+92%) than controls. Further ventriculo-cisternal perfusion studies showed that CSF Cu clearance by the choroid plexus is significantly greater in Fe-deficient animals than

controls. Thus, the authors suggested that the Fe-deficient condition has a profound effect on how the brain barrier systems transport Cu ions between the blood, brain interstitial fluid and CSF. More recent data from this group indicate that Fe deficiency leads to increased mRNA levels of DMT1, but not CTR1, suggesting a critical role of DMT1 in cellular Cu regulation during the Fe deficiency.

6. Conclusions

Both Fe and Cu are highly redox-reactive metals. A deficiency or excess of either metal in the CNS is detrimental to the brain's structure and function and can lead to oxidative stress and damage the mature neurons and neuronal activities. It is this delicate balance between good and evil that mammalian brains developed the precise mechanisms to maintain CNS metal ion homeostasis. The brain barrier system, i.e., the BBB and the BCB, plays a quintessential role in regulating Fe and Cu homeostasis in the brain. Current evidence suggests that both of these metals are transported into the brain primarily through the BBB; this process is accomplished by the regulation of metal uptake, intracellular trafficking, and export in the BBB endothelial cells. Perhaps this is a result of the similarity between the chemical properties of Cu and Fe. The luminal TfR-bound Fe ions are taken up by TfR-mediated endocytosis; a significant portion of free, unbound Fe is transported by either DMT1 or other yet-unidentified metal transporters. Free unbound Cu species are either reduced and/or taken up by CTR1, or DMT1. Both metals are released by the endothelial cells and pass into the brain interstitium to be utilized in neuronal activities. Excess Fe and Cu can flow into the CSF in brain ventricles, where the choroidal epithelial microvilli captures the metals. Fe is transported by DMT1, whereas Cu is taken up by both CTR1 and DMT1 in the BCB. The transport of both metals from the CSF against the concentration gradient to the blood suggests an active role of the BCB in regulating both metals in the CSF. Damaging the BBB-BCB regulatory pathways, either due to genetic alterations or environmental interferences, has been shown to result in clinically significant neurological diseases and disorders. Thus, understanding the regulation of Fe and Cu homeostasis in the CNS will aid in the design of new drugs targeted to the regulatory proteins at the brain barriers for the treatment of metal deficiency- or overload-related neurological diseases.

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References

Arredondo M, Munoz P, Mura CV, Nunez MT. DMT-1, a physiologically relevant apical Cu1+ transporter in intestinal cells. Am J Physiol Cell Physiol. 2003; 284:C1525–C1530. [PubMed: 12734107]

Ba LA, Doering M, Burkholz T, Jacob C. Metal trafficking: from maintaining metal homeostasis to future drug design. Metallomics. 2009; 1:292–311. [PubMed: 21305127]

Bali PK, Zak O, Aisen P. A new role for the transferrin receptor in the release of iron from transferrin. Biochemistry. 1991; 30:324–328. [PubMed: 1988034]

Barnham KJ, Masters CL, Bush AI. Neurodegenerative diseases and oxidative stress. Nat Rev Drug Discov. 2004; 3:204–214.

Bertinato J, Iskandar M, L'Abbe MR. Copper deficiency induces the upregulation of copper chaperone for Cu/Zn superoxide dismutase in weanling male rats. Journal of Nutrition. 2003; 133:28–31. [PubMed: 12514262]

Betarbet R, Sherer TB, MacKenzie C, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nature Neuroscience. 2000; 3:1301–1306.

Bohlken A, Cheung BB, Bell JL, Koach J, Smith S, Sekyere E, Thomas W, Norris M, Haber M, Lovejoy DB, Richardson DR, Marshall GM. ATP7A is a novel target of retinoic acid receptor beta. 2 in neuroblastoma cells. Br J Cancer. 2009; 100(1):96–105. [PubMed: 19127267]

Bradbury, M. The concept of a blood-brain barrier. Wiley; New York: 1979.

Bradbury M. An approach to study of transport of trace metals at the blood-brain barrier. Progr in Brain Res. 1992; 91:133–138.

Bressler JP, Olivi L, Cheong JH, Kim Y, Maerten A, Bannon D. Metal transporters in intestine and brain: their involvement in metal associated neurotoxicities. Hum Exp Tox. 2007; 26:221–229.

Brown DR. Copper and prion disease. Brain Res Bul. 2001; 55(2):165–173.

Burdo JR, Menzies SL, Simpson IA, Garrick LM, Garrick MD, Dolan KG, Haile DJ, Beard JL, Connor JR. Distribution of divalent metal transporter 1 and metal transport protein 1 in the normal and Belgrade rat. J Neurosci Res. 2001; 66:1198–1207. [PubMed: 11746453]

Cecchelli R, Berezowski V, Lundquist S, Culot M, Renftel M, Dehouck MP, Fenart L. Modelling of the blood-brain barrier in drug discovery and development. Nat Rev Drug Discov. 2007; 6(8):650–61. [PubMed: 17667956]

Choi BS, Zheng W. Copper transport to the brain by the blood-brain barrier and blood-CSF barrier. Brain Res. 2009; 1258:14–21. [PubMed: 19014916]

Choi CJ, Anantharam V, Martin DP, Nicholson EM, Richt JA, Kanthasamy AG. Manganese upregulates cellular prion protein and contributes to altered stabilization and proteolysis: relevance to role of metals in pathogenesis of prion disease. Toxicol Sci. 2010; 115(2):535–546. [PubMed: 20176619]

Collins JF, Hua P, Lu Y, Ranganathan PN. Alternative splicing of the Menkes copper Atpase (Atp7a) transcript in the rat intestinal epithelium. Am J Physiol Gastrointest Liver Physiol. 2009; 297(4):G695–707. [PubMed: 19679821]

Collins J. Gene chip analyses reveal differential genetic responses to iron deficiency in rat duodenum and jejunum. Biol Res. 2006; 39(1):25–37. [PubMed: 16629162]

Crichton RR, Dexter DT, Ward RJ. Brain iron metabolism and its perturbation in neurological diseases. J Neural Transm. 2011; 118:301–314. [PubMed: 20809066]

Crossgrove JS, Zheng W. Manganese toxicity upon overexposure. NMR in Biomedicine. 2004; 17(8): 544–553. [PubMed: 15617053]

Crossgrove JS, Li GJ, Zheng W. The choroid plexus removes beta-amyloid from the cerebrospinal fluid. Exp Biol Med. 2005; 230(10):771–776.

Crowe A, Morgan EH. Iron and copper interact during their uptake and deposition in the brain and other organs of developing rats exposed to dietary excess of the two metals. J Nutr. 1996; 126:183–194. [PubMed: 8558300]

Deane R, Zheng W, Zlokovic BV. Brain capillary endothelium and choroid plexus epithelium regulate transport of transferrin-bound and free iron into the rat brain. J Neurochem. 2004; 88:813–820. [PubMed: 14756801]

Dexter DT, Wells FR, Agid F, Agid Y, Lees AJ, Jenner P, Marsden CD. Increased nigral iron content in postmortem parkinsonian brain. Lancet. 1987; 2:1219–1220. [PubMed: 2890848]

Fairweather-Tait SJ. Iron nutrition in the UK: getting the balance right. Proc Nutr Soc. 2004; 63:519–528. [PubMed: 15831123]

Ferenci P. Review article: diagnosis and current therapy of Wilson's disease. Aliment Pharmacol Ther. 2004; 19:157–165. [PubMed: 14723607]

Ferguson CJ, Wareing M, Ward DT, Green R, Smith CP, Riccardi D. Cellular localization of divalent metal transporter DMT-1 in rat kidney. Am J Physiol Renal Physiol. 2001; 280:F803–F814. [PubMed: 11292622]

Forte G, Bocca B, Senofonte O, Petrucci F, Brusa L, Stanzione P, Zannino S, Violante N, Alimonti A, Sancesario G. Trace and major elements in whole blood, serum, cerebrospinal fluid and urine of patients with Parkinson's disease. J Neural Transmission. 2004; 111(8):1031–1040.

Gaggelli E, Kozlowski H, Valensin D, Valensin G. Copper homeostasis and neurodegenerative disorders (Alzheimer's, prion, and Parkinson's diseases and amyotrophic lateral sclerosis). Chem Rev. 2006; 106(6):1995–2044. [PubMed: 16771441]

Gambling L, McArdle HJ. Iron, copper, and fetal development. Proc of the Nutr Soc. 2004; 63:553–562.

Garrick MD, Dolan KG, Horbinski C, Ghio AJ, Higgins D, Porubcin M, Moore EG, Hainsworth LN, Umbreit JN, Conrad ME, Feng L, Lis A, Roth JA, Singleton S, Garrick LM. DMT1: a mammalian transporter for multiple metals. Biometals. 2003; 16(1):41–54. [PubMed: 12572663]

Gellein K, Roos PM, Evje L, Vesterberg O, Flaten TP, Nordberg M, Syversen T. Separation of proteins including metallothionein in cerebrospinal fluid by size exclusion HPLC and determination of trace elements by HR-ICP-MS. Brain Res. 2007; 1174:136–142. [PubMed: 17868660]

Georgieff MK, Wobken JK, Welle J, Burdo JR, Connor JR. Identification and localization of divalent metal transporter-1 (DMT-1) in term human placenta. Placenta. 2000; 21:799–804. [PubMed: 11095929]

Gitlin JD. Wilson disease. Gastroenterology. 2003; 125:1868–1877. [PubMed: 14724838]

Gotz ME, Double K, Gerlach M, Youdim MB, Riederer P. The relevance of iron in the pathogenesis of Parkinson's disease. Ann N Y Acad Sci. 2004; 1012:193–208. [PubMed: 15105267]

Gruenheid S, Cellier M, Vidal S, Gros P. Identification and characterization of second mouse Nramp gene. Genomics. 1995; 25:514–525. [PubMed: 7789986]

Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL, Hediger MA. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature. 1997; 388:482–488. [PubMed: 9242408]

Gunshin H, Allerson CR, Polycarpou-Schwarz M, Rofts A, Rogers JT, Kishi F, Hentze MW, Rouault TA, Andrews NC, Hediger MA. Iron-dependent regulation of the divalent metal ion transporter. FEBS Let. 2001; 509:309–316. [PubMed: 11741608]

Gybina AA, Prohaska JR. Variable response of selected cuproproteins in rat choroid plexus and cerebellum following perinatal copper deficiency. Genes Nutr. 2006; 1(1):51–60. [PubMed: 18850220]

Han O, Wessling-Resnick M. Copper repletion enhances apical iron uptake and transepithelial iron transport by Caco-2 cells. Am J Physiol Gastrointest Liver Physiol. 2002; 283(3):G527–G533. [PubMed: 11842003]

Harris ED. Copper homeostasis: the role of cellular transporters. Nutr Rev. 2001; 59:281–285. [PubMed: 11570430]

He Y, Thong PS, Lee T, Leong SK, Shi CY, Wong PT, Yuan SY, Watt F. Increased iron in the substantia nigra of 6-OHDA induced parkinsonian rats: a nuclear microscopy study. Brain Res. 1996; 735:149–53. [PubMed: 8905180]

Huang E, Ong WY, Connor JR. Distribution of divalent metal transporter-1 in the monkey basal ganglia. Neuroscience. 2004; 128:487–496. [PubMed: 15381278]

Jiang YM, Zheng W, Long LL, Zhao WJ, Li XG, Mo XA, Lu JP, Fu X, Li WM, Liu SF, Long QY, Huang JL, Pira E. Brain magnetic resonance imaging and manganese concentrations in red blood cells of smelting workers: Search for biomarkers of manganese exposure. NeuroToxicology. 2007; 28:126–135. [PubMed: 16978697]

Kaler SG. ATP7A-related copper transport diseases-emerging concepts and future trends. Nature Rev Neurol. 2011; 7:15–29. [PubMed: 21221114]

Ke Y, Chen YY, Chang YZ, Duan XL, Ho KP, Jiangde H, Wang K, Qian ZM. Post-transcriptional expression of DMT1 in the heart of rat. J Cell Physiol. 2003; 196:124–130. [PubMed: 12767048]

Keep RF, Jones HC. A morphometric study on the development of the lateral ventricle choroid plexus, choroid plexus capillaries and ventricular ependyma in the rat. Develop Brain Res. 1990; 56:47–53.

Kell DB. Towards a unifying, systems biology understanding of large-scale cellular death and destruction caused by poorly liganded iron: Parkinson's, Huntington's Alzheimer's, prirons, bactericides, chemical toxicology and others as examples. Arch Toxicol. 2010; 84:825–889. [PubMed: 20967426]

Kuo YM, Gybina AA, Pyatskowit JW, Gitschier J, Prohaska JR. Copper transport protein (Ctr1) levels in mice are tissue specific and dependent of copper status. J Nutr. 2006; 136:21–26. [PubMed: 16365053]

LaVaute T, Smith S, Cooperman S, Iwai K, Land W, Meyron-Holtz E, Drake SK, Miller G, Abu-Asa M, Tsoko M, Switzer R, Grinberg A, Love P, Tresser N, Rouault TA. Targeted deletion of the gene encoding iron regulatory protein-2 causes misregulation of iron metabolism and neurodegenerative disease in mice. Nature Gent. 2001; 27:209–214.

Lee PL, Gelbert T, West C, Halloran C, Beutler E. The human Nramp2 gene: characterization of the gene structure, alternative splicing, promoter region and poly morphisms. Blood Cells Mol Dis. 1998; 24:199–215. [PubMed: 9642100]

Lee J, Prohaska JR, Thiele DJ. Essential role for mammalian copper transporter Ctr1 in copper homeostasis and embryonic development. Proc Natl Acad Sci USA. 2001; 98:6842–6847. [PubMed: 11391005]

Li GJ, Zhao Q, Zheng W. Alteration at translational but not transcriptional level of transferrin receptor expression following manganese exposure at the blood–CSF barrier in vitro. Toxicol Appl Pharmacol. 2005; 205:188–200. [PubMed: 15893546]

Li GJ, Choi BS, Wang X, Liu J, Waalkes MP, Zheng W. Molecular mechanism of distorted iron regulation in the choroids plexus and selected brain regional capillaries following in vivo manganese exposure. NeuroToxicology. 2006; 27:737–744. [PubMed: 16545456]

Li X, Chen S, Wang Q, Zack DJ, Snyder SH, Borjigin J. A pineal regulatory element (PIRE) mediates transactivation by the pineal/retina-specific transcription factor CRX. Proc Natl Acad Sci USA. 1998; 95(4):1876–1881. [PubMed: 9465110]

Linder, MC. Biochemistry of Copper. Plenum Press; New York: 1991.

Linder MC, Hazegh-Azam M. Copper biochemistry and molecular biology. American J Clin Nutr. 1996; 63:797S–811S.

Liu PC, Chen YW, Centeno JA, Quezado M, Lem K, Kaler SG. Downregulation of myelination, energy, and translational genes in Menkes disease brain. Mol Genet Metab. 2005; 85:291–300. [PubMed: 15923132]

Loudianos G, Lovicu M, Dessi V, Tzetis M, Kanavakis E, Zancan L, Zelante L, Galvez-Galvez C, Cao A. Abnormal mRNA splicing resulting from consensus sequence splicing mutations of ATP7B. Human Mutation. 2002; 20(4):260–266. [PubMed: 12325021]

Lu L, Zhang LL, Li GJ, Guo W, Liang W, Zheng W. Serum concentrations of manganese and iron as the potential biomarkers for manganese exposure in welders. NeuroToxicology. 2005; 26(2):257–265. [PubMed: 15713346]

Lutsenko S, Petris MJ. Function and regulation of the mammalian copper-transporting ATPases: insights from biochemical and cell biological approaches. J Membr Biol. 2003; 191:1–12. [PubMed: 12532272]

Lutsenko S, Barnes NL, Bartee MY, Dmitriev OY. Function and Regulation of Human Copper-Transporting ATPases. Physiol Rev. 2007; 87:1011–1046. [PubMed: 17615395]

Lutsenkno S, Bhattacharjee A, Hubbard AL. Copper handling machinery of the brain. Metallomics. 2010; 2:596–608. [PubMed: 21072351]

Marques F, Falcao AM, Sousa JC, Coppola G, Geschwind D, Sousa N, Correia-Neves M, Palha JA. Altered iron metabolism is part of the choroid plexus response to peripheral inflammation. Endocrinology. 2009; 150:2822–2828. [PubMed: 19213835]

Martin DP, Anantharam V, Jin H, Witte T, Houk R, Kanthasamy A, Kanthasamy AG. Infectious prion protein alters manganese transport and neurotoxicity in a cell culture model of prion disease. Neurotoxicology. 2011 Aug 19. [Epub ahead of print].

Monnot AD, Behl M, Ho S, Zheng W. Regulation of brain copper homeostasis by the brain barrier system: Effects of Fe-overload and Fe-deficiency. Toxicol Appl Pharmacol. 2011; 256:249–257. [PubMed: 21315754]

Moos T, Rosengren-Nielsen T. Ferroportin in the postnatal rat brain: implications for axonal transport and neuronal export of iron. Semin Pediatr Neurol. 2006; 13:49–57.

Moos T, Skjoerringe T, Gosk S, Morgan EH. Brain capillary endothelial cells mediate iron transport into the brain by segregating iron from transferrin without the involvement of divalent metal transporter 1. J Neurochem. 2006; 98:1946–1958. [PubMed: 16879716]

Moos T, Rosengren-Nielsen T, Skjørringe T, Morgan EH. Iron trafficking inside the brain. J Neurochem. 2007; 103:1730–1740. [PubMed: 17953660]

Morris CM, Candy JM, Oakley AE, Bloxham CA, Edwardson JA. Histochemical Distribution of Non-Haem Iron in the Human Brain. Acta Anatomica. 1992; 144:235–257. [PubMed: 1529678]

Nischwitz V, Berthele A, Michalke B. Speciation analysis of selected metals and determination of their total contents in paired serum and cerebrospinal fluid samples: An approach to investigate the permeability of the human blood-cerebrospinal fluid-barrier. Analytica Chimica Acta. 2008; 627(2):258–269. [PubMed: 18809082]

Nishihara E, Furuyama T, Yamashita S, Mori N. Expression of copper trafficking genes in the mouse brain. Neuroreport. 1998; 9:3259–3263. [PubMed: 9831461]

Nishimura N, Nishimura H, Ghaffar A, Tohyama C. Localization of metallothionein in the brain of rat and mouse. J Histochem Cytochem. 1992; 40:309–315. [PubMed: 1552172]

Opazo C, Huang X, Chemy RA, Moir RD, Roher AE, White AR, Cappai R, Masters CL, Tanzi RE, Inestrosa NC, Bush AI. Metalloenzyme-like activity of Alzheimer's disease beta-amyloid. Cudependent catalytic conversion of dopamine, cholesterol, and biological reducing agents to neurotoxic H_2O_2. J Biol Chem. 2002; 277:40302–40308. [PubMed: 12192006]

Pardridge WM. Comparison of in vitro and in vivo models of drug transcytosis through the blood-brain barrier. J Pharmacol Exp Ther. 1990; 253:884–891. [PubMed: 2338660]

Pardridge, WM. Peptide drug delivery to the brain. Raven Press; New York: 1991.

Pauly PC, Harris DA. Copper stimulates endocytosis of the prion protein. J Biol Chem. 1998; 273(50): 33107–33110. [PubMed: 9837873]

Phinney AL, Drisaldi B, Schmidt SD, Lugowski S, Coronado V, Liang Y, Horne P, Yang J, Sekoulidis J, Coomaraswamy J, Chishti MA, Cox DW, Mathews PM, Nixon RA, Carlson GA, St George-Hyslop P, Westaway D. In vivo reduction of amyloid-beta by a mutant copper transporter. Proc Natl Acad Sci USA. 2003; 100:14193–14198. [PubMed: 14617772]

Poduslo JF, Whelan SL, Curran GL, Wengenack TM. Therapeutic benefict of polyamine-modified catalase as a scavenger of hydrogen peroxide and nitric oxide of familial amyotrophic lateral sclerosis transgenics. Ann Neurol. 2000; 48:943–947. [PubMed: 11117554]

Qian Y, Mikeska G, Harris ED, Bratton GR, Tiffany-Castiglioni E. Effect of lead exposure and accumulation on copper homeostasis in cultured C6 rat glioma cells. Toxicol Appl Pharmacol. 1999; 158:41–49. [PubMed: 10387931]

Redzic Z. Molecular biology of the blood-brain and the blood-cerebrospinal fluid barriers: similarities and differences. Fluids Barriers CNS. 2011; 8(3):1–25. [PubMed: 21349146]

Roth JA, Garrick MD. Iron interactions and other biological reactions mediating the physiological and toxic actions of manganese. Biochem Pharmacol. 2003; 66:1–13. [PubMed: 12818360]

Roth JA, Horbinski C, Feng L, Dolan KG, Higgins D, Garrick MD. Differential localization of divalent metal transporter 1 with and without iron response element in rat PC12 and sympathetic neuronal cells. J Neurosci. 2000; 20:7595–7601. [PubMed: 11027219]

Rouault TA. The role of iron regulatory proteins in mammalian iron homeostasis and disease. Nat Chem Biol. 2006; 2:406–414. [PubMed: 16850017]

Rouault TA, Zhang DL, Jeong SY. Brain iron homeostasis, the choroid plexus, and localization of iron transport proteins. Metab Brain Dis. 2009; 24:673–684. [PubMed: 19851851]

Salustri C, Barbati G, Ghidoni R, Quintiliani L, Ciappina S, Binetti G, Squitti R. Is cognitive function linked to serum free copper levels ? A cohort study in a normal population. Clin Neurophysiol. 2010; 121(4):459–460. [PubMed: 20071223]

Sastry S, Arendash GW. Time-dependent changes in iron levels and associated neuronal loss within the substantia nigra following lesions within the neostriatum/globus pallidus complex. Neuroscience. 1995; 67:649–666. [PubMed: 7545796]

Sharp P. The molecular basis of copper and iron interactions. Proc of the Nutr Soc. 2004; 63:563–569.

Siddappa AJ, Rao RB, Wobken JD, Leibold EA, Connor JR, Georgieff MK. Developmental changes in the expression of iron regulatory proteins and iron transport proteins in the perinatal rat brain. J Neurosci Res. 2002; 68:761–775. [PubMed: 12111837]

Sipe DM, Murphy RF. Binding to cellular receptor results in increased iron release from transferrin at mildly acidic pH. J Biol Chem. 1991; 266:8002–8007. [PubMed: 2022630]

Sofic E, Riederer P, Heinsen H, Beckmann H, Reynolds GP, Hebenstreit G, Youdim MB. Increased iron (III) and total iron content in post mortem substantia nigra of parkinsonian brain. J Neural Transm. 1988; 74:199–205. [PubMed: 3210014]

Sparks DL, Schreurs BG. Trace amounts of copper in water induce β-amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease. Proc Natl Acad Sci USA. 2003; 100(19):11065–11069. [PubMed: 12920183]

Speake T, Brown PD. Ion channels in epithelial cells of the choroid plexus isolated from the lateral ventricle of rat brain. Brain Res. 2004; 1005:60–66. [PubMed: 15044065]

Stuerenburg HJ. CSF copper concentrations, blood-brain barrier function, and ceruloplasmin synthesis during the treatment of Wilson's disease. J Neural Transm. 2000; 107:321–329. [PubMed: 10821440]

Sugawara N, Ikeda T, Sugawara C, Kohgo Y, Kato J, Takeichi N. Regional distribution of copper, zinc and iron in the brain in Long-Evans Cinnamon (LEC) rats with a new mutation causing hereditary hepatitis. Brain Res. 1992; 588:287–290. [PubMed: 1393580]

Uchino M, Ando Y, Tanaka Y, Nakamura T, Uyama E, Mita S, Murakami T, Ando M. Decrease in Cu/Zn- and Mn-superoxide dismutase activities in brain and spinal cord of patients with amyotrophic lateral sclerosis. J Neurol Sci. 1994; 127:61–67. [PubMed: 7699393]

Wang X, Li GJ, Zheng W. Up regulation of DMT1 expression in choroidal epithelia of the blood-CSF barrier following manganese exposure in vivo. Brain Res. 2006; 1097:1–10. [PubMed: 16729984]

Wang XQ, Miller DS, Zheng W. Intracellular trafficking of metal transporters in intact rat choroid plexus following in vitro treatment of manganese or iron. Toxicol Appl Pharmacol. 2008a; 230:167–174. [PubMed: 18420243]

Wang X, Li GJ, Zheng W. Efflux of iron from the cerebrospinal fluid to the blood at the blood-CSF barrier: effect of manganese exposure. Exp Biol Med. 2008b; 233(12):1561–1571.

Wu LJ, Leenders AG, Cooperman S, Meyron-Holtz E, Smith S, Land W, Tsai RY, Berger UV, Sheng ZH, Rouault TA. Expression of the iron transporter ferroportin in synaptic vesicles and the blood-brain barrier. Brain Res. 2004; 1001:108–117. [PubMed: 14972659]

Yang WM, Jung KJ, Lee MO, Lee YS, Lee YH, Nakagawa S, Niwa M, Cho SS, Kim DW. Transient expression of iron transport proteins in the capillary of the developing rat brain. Cel Mol Neurobiol. 2011; 31:93–99.

Yokel RA. Blood-brain barrier flux of aluminum, manganese, iron and other metals suspected to contribute to metal-induced neurodegeneration. J Alzheimer's Dis. 2006; 10:223–253. [PubMed: 17119290]

Yoshimura Y, Kida K, Usutani S, Nishimura M. Histochemical localization of copper in various organs of brindled mice after copper therapy. Pathol Int. 1995; 45:10–18. [PubMed: 7704239]

Zecca L, Youdim MB, Riederer P, Connor JR, Crichton RR. Iron, brain ageing and neurodegenerative disorders. Nat Rev Neurosci. 2004; 5(11):863–873. [PubMed: 15496864]

Zheng W. Neurotoxicology of the brain barrier system: New implications. J Toxicol – Clin Toxicol. 2001; 39(7):711–719. [PubMed: 11778669]

Zheng, W. Introduction to the blood-CSF barrier. In: Zheng, W.; Chodobski, A., editors. The Blood-Cerebrospinal Barrier. CRC Press; New York: 2005. p. 3-7.

Zheng W, Ren S, Graziano JH. Manganese inhibits mitochondrial aconitase: a mechanism of manganese neurotoxicity. Brain Res. 1998; 799:334–342. [PubMed: 9675333]

Zheng W, Zhao Q, Slavkovich V, Aschner M, Graziano H. Alteration of iron homeostasis following chronic exposure to manganese in rats. Brain Res. 1999; 833:125–132. [PubMed: 10375687]

Zheng W, Aschner M, Ghersi-Egea J. Brain barrier systems: a new frontier in metal neurotoxicological research. Toxicol Appl Pharmacol. 2003; 192:1–11. [PubMed: 14554098]

Zheng W, Fu X, Dydak U, Cowan DM. Biomarkers of manganese intoxication. NeuroToxicology. 2011; 32(1):1–8. [PubMed: 20946915]

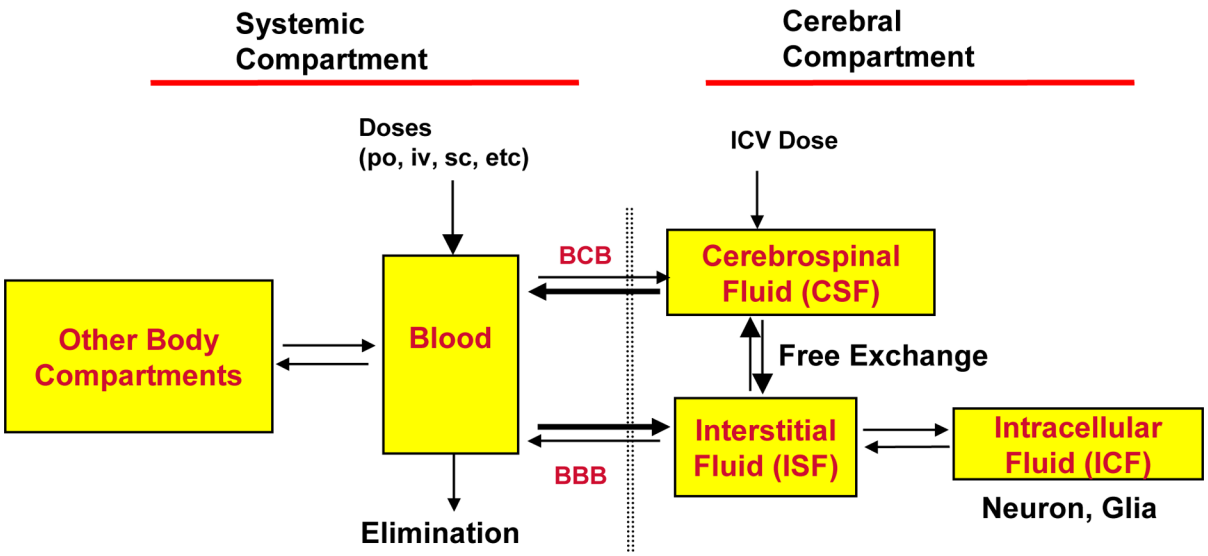

Fig. 1.

Brain Fluid Compartments and Brain Barrier Systems. Substances in the blood can pass across the blood-brain barrier (BBB) to enter the brain interstitial fluid (ISF) and come into contact with neuronal structures. Substances in the blood can also pass across the blood-CSF barrier (BCB) to enter the cerebrospinal fluid (CSF). Since there is no structural barrier between the CSF and ISF, materials in these two fluid compartment can freely exchange. The intra-cerebroventricular (ICV) injection of toxic chemicals produces more severe neurotoxicity than do other routes of exposure, suggesting a direct delivery of chemicals from the CSF to the ISF. Metals such as Fe and Cu may enter the ISF via the BBB and be transported back into the blood via the efflux mechanism at the BCB.

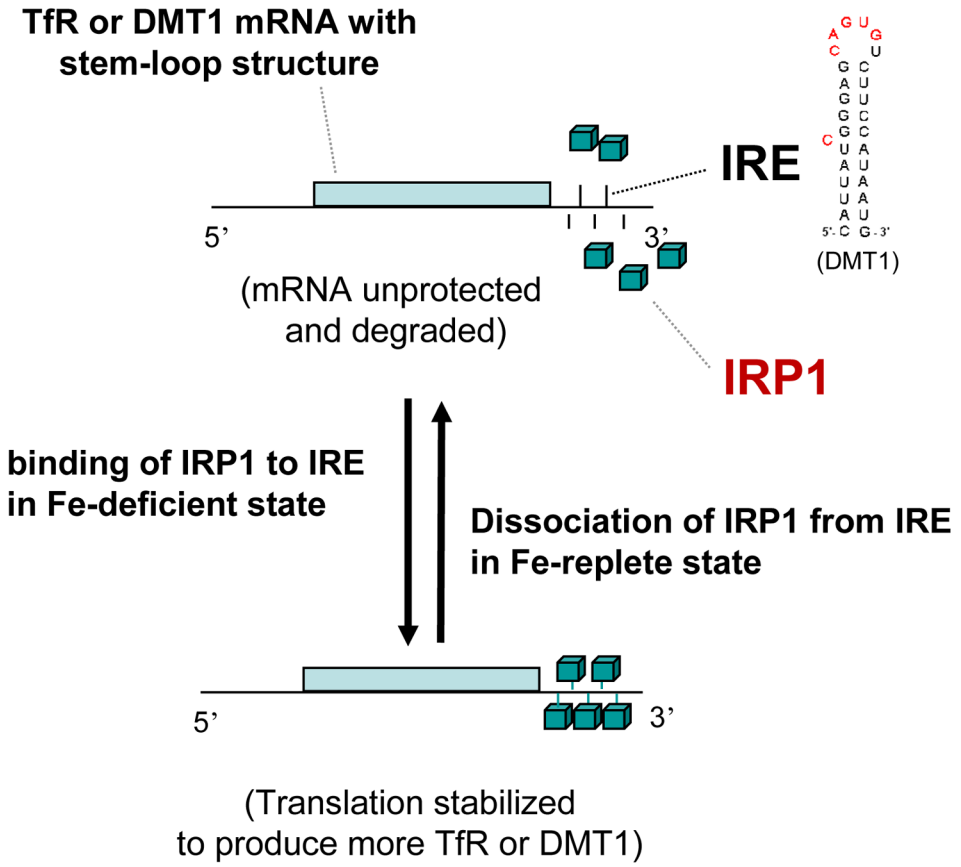

Fig. 2.

Iron Regulatory Protein-1 (IRP1) and Intracellular Fe Regulation. mRNAs encoding transferrin receptor (TfR) and divalent metal transporter (DMT1) possess the stem-loop structure called the iron responsive element (IRE) at the 3′ untranslated region. In the Fe deficient state, the conformation change in the [4Fe-4S] active center in IRP1 allows the IPR1 to bind to the stem loops of TfR or DMT1 mRNAs; the binding stabilizes the translation, produces more TfR or DMT1, and allows the cell to take up more Fe to meet the metabolic needs.

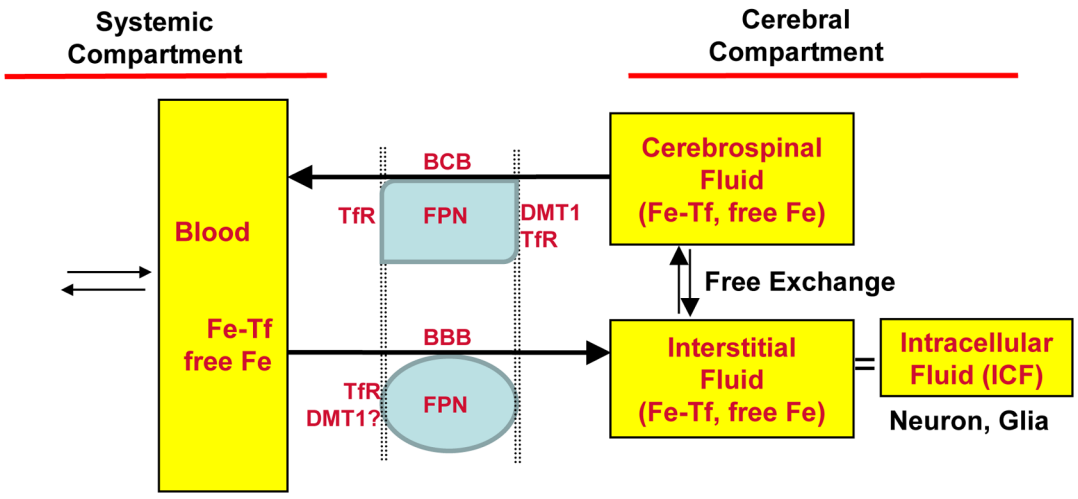

Fig. 3.

Iron Transport by the BBB and BCB. Most Fe molecules in the blood are bound to Tf. At the BBB, Fe-Tf is transported into the cerebral endothelial cells by TfR; a portion of free Fe ions are transported via DMT1 or other yet-identified transporters. The FPN exports Fe into the interstitial fluid, where Fe is utilized by cells. Excess Fe ions in the CSF are taken up by DMT1 in choroidal epithelial microvilli and transported back to the blood.

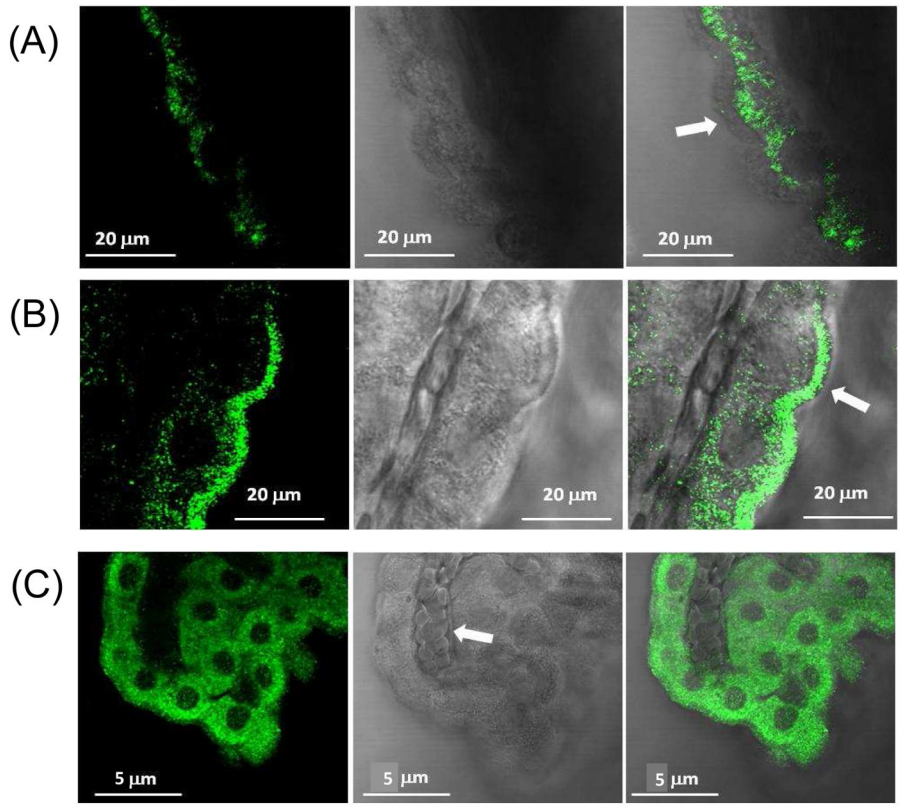

Fig. 4.

Intracellular Distribution of CTR1 and ATP7A in Choroid Plexus by Confocal Microscopy. (A). Presence of CTR1 in rat choroid plexus. CTR1 signals are cytosol-distributed mainly between the nuclei and apical side membrane. The arrow indicates the apical microvilli without CTR1 staining. (B). Subchronic exposure to Mn in rats causes the relocation of CTR1 to the apical brush board of choroidal epithelium. The arrow indicates a substantial staining in the apical microvilli. (C). Presence of ATP7A in rat choroid plexus. ATP7A signals are evenly distributed in the cytosol. The arrow indicates red blood cells in choroidal capillary vessel. Left: immunohistochemical staining; Middle: transmission image; Right: imaging overlay.

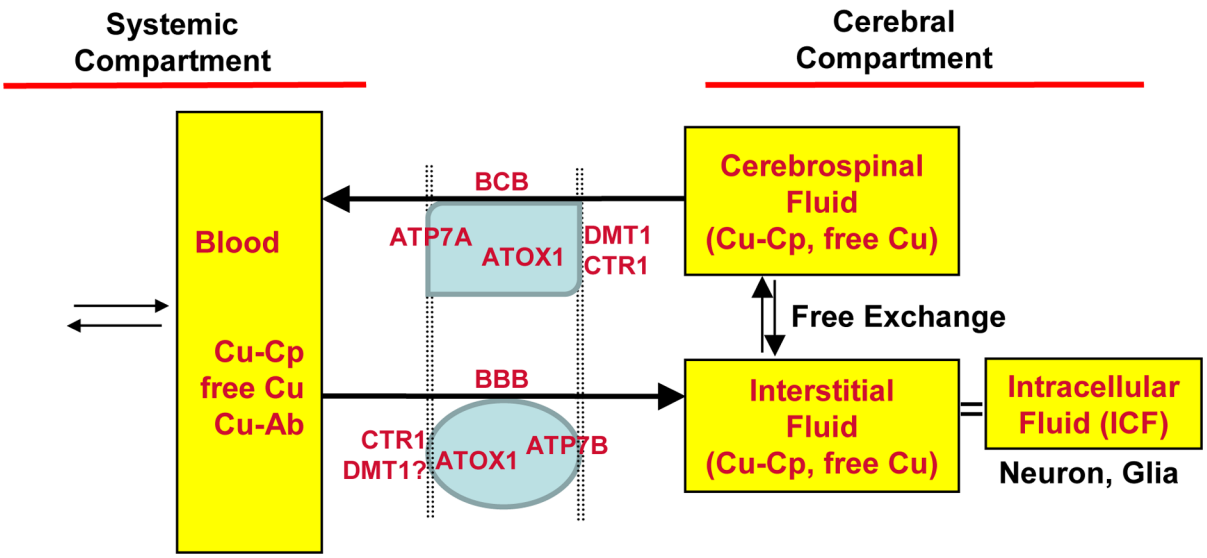

Fig. 5.

Cu Transport by the BBB and BCB. Most of the Cu molecules in the blood are bound to ceruloplasmin (Cp). At the BBB, free Cu is transported into the cerebral endothelial cells by CTR1; a portion of free Cu ions are transported via DMT1 or other yet-identified transporters. ATOX1 delivers Cu to either ATP7A or ATP7B to be released into the interstitial fluid, where Cu is utilized by neurons and neuroglial cells. Excess Cu ions in the CSF are taken up by CTR1 or DMT1 in choroidal epithelial microvilli and transported back to the blood.

Table 1

Major Proteins Involving Iron Regulation

Name and Abbreviation	Fe Species bound	Presence in				Function
		BBB	BCB	CSF	Blood	
Transferrin (Tf)	Fe(III)			+	+	Fe carrier
Transferrin receptor (TfR)	Fe(II)	+	+	?	+	cell surface Fe uptake
Ferritin (Ft)	Fe(II)	+	+	?	+	Fe storage
Iron Regulatory Proteins (IRPs)		+	+	?	+	regulates the expression of Tf, TfR, DMT1, FPN
Divalent Metal Transporter (DMT1)	Fe(II)	?	+			cell surface Fe uptake
Ferroportin (FPN, MTP1)	Fe(II)	+	+			cellular Fe exporter
Hephaestin	Fe(II)	+	+			oxidize Fe(II) to Fe(III)
Steap3	Fe(III)	+	?			reduce Fe(III) to Fe (II)
Hepcidin		+	+			regulate FPN expression

+: identified;

?: uncertain.

General source: Kell, 2010; Li, 2006; Lu et al., 2005; Rouault et al., 2009; Wang et al., 2008a,b. Hephaestin and MTP1 in BBB: Yang et al., 2011. Hepcidin in BBB and BCB: Crichton et al., 2011; Marques et al., 2009. Lactoferrin: Moos et al., 2007.

Table 2

Major Proteins Involving Copper Regulation

Name and Abbreviation	Cu Species bound	Presence in				Function
		BBB	BCB	CSF	Blood	
Ceruloplasmin	Cu(I)			+	+	Cu carrier in plasma and ferroxidase
Copper Transporter-1 (CTR1)	Cu(I)	+	+			cell surface Cu uptake
Divalent Metal Transporter (DMT1)	Cu(II)	?	+			cell surface Cu uptake
Iron Regulatory Proteins (IRPs)		+	+		+	regulates expression of DMT1
ATP7A	Cu(I)	+	+			cellular Cu exporter
ATP7B	Cu(I)	+	+			cellular Cu exporter
Antioxidant Protein 1 (ATOX1)	Cu(I)	?	+			intracellular Cu chaperone
Cu chaperone SOD (CCS)	Cu(I)	?	+			intracellular Cu chaperone
Cyto oxidase complex (COX17)	Cu(I)	?	?	?	?	intracellular Cu chaperone
Steap2	Cu(II)	+				reduce Cu(II) to Cu (I)

+ : identified.

?: uncertain.

Source: Lutsenko et. al, 2010; Choi et al., 2009.