



Associate editor: C. Pope

## Regulation of brain iron and copper homeostasis by brain barrier systems: Implication in neurodegenerative diseases

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### ARTICLE INFO

#### Keywords:

Iron  
Copper  
Blood–brain barrier  
Blood–CSF barrier  
Choroid plexus  
Transferrin receptor or TfR  
Divalent metal transporter or DMT1  
Copper transporter or CTR1

### 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.

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### 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 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

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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  $\beta$ -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 composed 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 and 20 m<sup>2</sup> (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 & Jones, 1990; Speake & 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

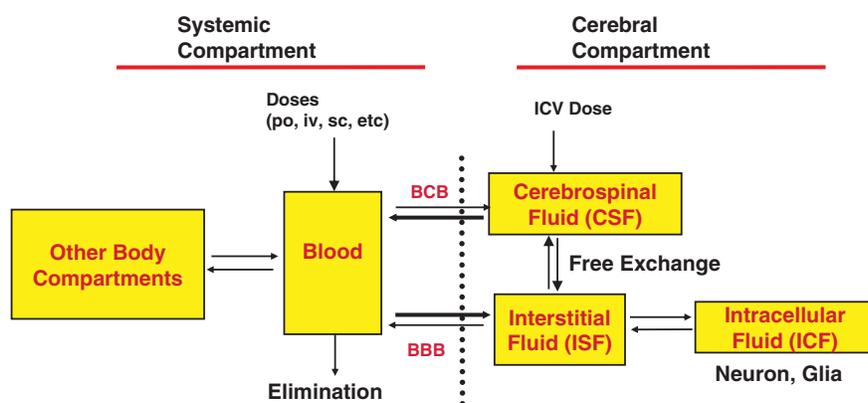
### 3.1. Systemic Fe homeostasis and regulation

The human body has an average of 4–5 g 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 are regulated by several macromolecules summarized in Table 1.

Once Fe enters the circulation from the small intestine, it binds to the  $\beta$ -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 Da) 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; Jefferies et al., 1984; Wang et al., 2008, 2009).



**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 compartments 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.

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 (Wu et al., 2004; Moos et al., 2007). 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  $^{59}\text{Fe}$ –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  $^{59}\text{FeCl}_3$  (Malecki et al. 1999). Takeda et al. (2002) also found the evidence of non-Tf mediated brain Fe uptake, when they intravenously injected  $^{59}\text{FeCl}_3$  into a Tf-saturated mouse model and discovered that  $^{59}\text{Fe}$  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  $^{59}\text{Fe}$ , 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 & Murphy, 1991). Presumably, 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

**Table 1**

Major proteins involving iron regulation.

General source: Kell, 2010; Li et al., 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.

	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.

been identified in duodenal epithelia, erythrocytes, renal epithelial cells, cardiomyocytes and human placenta epithelia (Georgieff et al., 2000; Roth et al., 2000; Ferguson et al., 2001; Ke et al., 2003). 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. Siddappa 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 & Garrick, 2003). Moreover, a high expression of DMT1 in 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 & Rosengren-Nielsen, 2006). 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, Hephidin) 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 (Address et al., 1997; Eisenstein and Ross, 2003; Konarska and Sharp, 1986; Rouault, 2006). 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 (Zheng et al., 1998; Li et al., 2005, 2006). 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 toward 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 & 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 (Zheng et al., 2003; Crossgrove et al., 2005; Redzic, 2011). 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.



out of the cell (Bressler et al., 2007; Choi & Zheng, 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 & Zheng, 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 and Zheng (2009) prepared three Cu species, i.e., free  $^{64}\text{Cu}$ ,  $^{64}\text{Cu}$ -albumin, and  $^{64}\text{Cu}$ -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  $\mu\text{M}$  or  $22.3 \pm 2.2 \mu\text{g/L}$ . Serum Cu concentration ( $1129 \pm 124 \mu\text{g/L}$ ) 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  $\text{K}^+$  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 (Gaggelli 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 (Kuo et al., 2006; Choi & Zheng, 2009). 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 (Han & Wessling-Resnick, 2002; Collins, 2006). Additionally, experimental evidence has shown that an Fe-deficient condition can increase intestinal tissue Cu levels and enhance the expression of both DMT1 and ATP7A in the intestine (Collins, 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, 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, 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 & Prohaska, 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 composed 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 ( $>10^{19} \text{M}^{-1}$  at pH 7.4) relative to zinc (Zn) ( $3.2 \times 10^{13} \text{M}^{-1}$ ) (Ba et al., 2009). As a result of the higher Cu-MT

**Table 2**

Major proteins involving copper regulation.

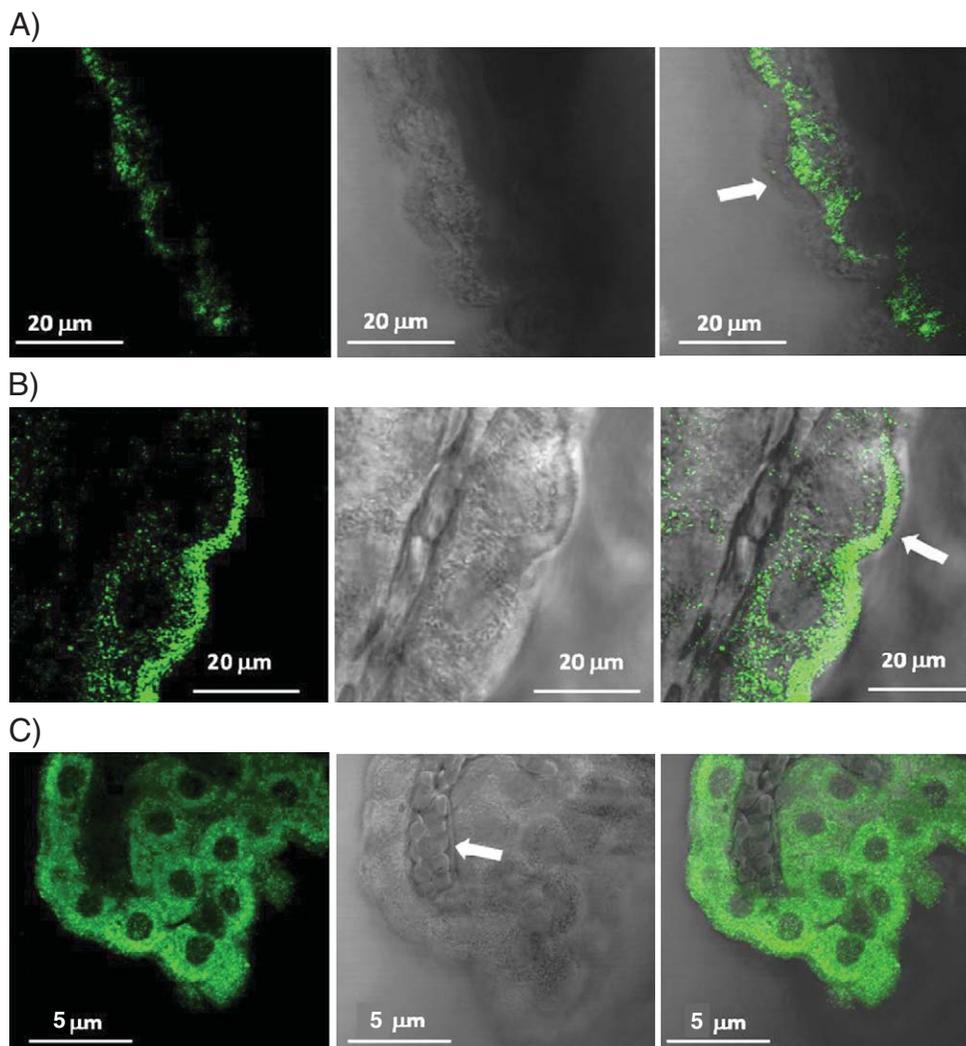
Source: Lutsenkno et al., 2010 Choi &amp; Zheng, 2009.

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.

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.



**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.

#### 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 (Nishihara et al., 1998; Choi & Zheng, 2009), 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 & Zheng, 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 is 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  $\beta$  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 (Loudianos et al., 2002; Collins et al., 2009). 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 and Zheng (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.

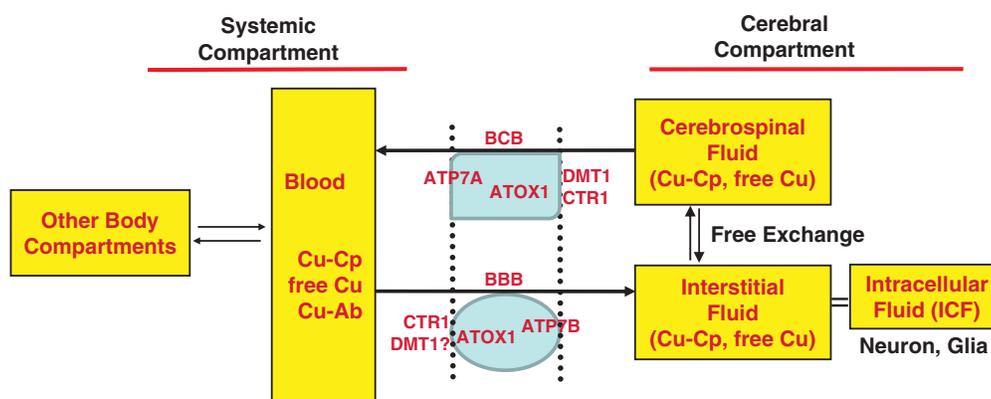
To address the directional transport of Cu by the BCB, Monnot et al. (2011) used a two-chamber Transwell culture system with the primary choroidal epithelial cells to compare the rate of Cu transport between the blood and CSF. The data demonstrated that the rate of Cu transport from the CSF to the blood 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 (Fig. 5). The free, unbound Cu ions are likely taken up by CTR1 in cerebral endothelia, and 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; Zecca et al., 2004; Kell, 2010). 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–



**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 is 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.

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 & Petris, 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 result 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 (Stuerenburg, 2000). These include liver dysfunction or failure, movement disorders, and psychiatric disturbances (Gitlin, 2003; Ferenci, 2004; Lutsenko et al., 2007).

### 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 (Pauly & Harris, 1998; Brown, 2001). 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  $\beta$ -amyloid plaques in a rabbit model of Alzheimer's disease (Sparks & Schreurs, 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  $\beta$ -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 & 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 (Sastry & Arendash, 1995; He et al., 1996). 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 & 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 (Zheng et al., 1999; Li et al., 2005, 2006). 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 remains 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 DMT1 mRNAs and in 5'-UTR of FPN mRNA (Zheng et al., 1998; Li et al., 2005, 2006). 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 & 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, 2004; Gambling & McArdle, 2004; Sharp, 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.

## Acknowledgment

The research in the Zheng laboratory has been supported in part by NIH/National Institute of Environmental Health Sciences Grants R01-ES008146-14, R21-ES013118, R21-ES017055, U.S. Department of Defense Contract USAMRMC W81XWH-05-1-0239, Eli Lilly Pharmaceuticals Grant RPFA # 0GJ63, and Johnson & Johnson Corp. Focused Research Given Award 2003111191. Dr. Monnot was partially supported by a Ross Fellowship from the Purdue Research Foundation during his doctoral research in School of Health Sciences at Purdue University.

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